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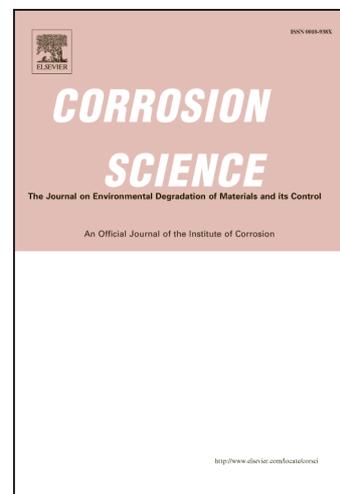
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Filtration-UV irradiation as an option for mitigating the risk of microbiologically influenced corrosion of subsea construction alloys in seawater

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Abstract

The effect of filtration-UV irradiation of seawater on the biofilm activity on several offshore structural alloys was evaluated in a continuous flow system over 90 days. Biofilms ennobled the electrode potential by +400-500 mV within a few days of exposure to raw untreated seawater. Filtration-UV irradiation of the seawater delayed the ennoblement of the steels for up to 40 days and lowered localized corrosion rates in susceptible alloys. Ennobling biofilms were composed of microbial cells, diatoms and extracellular polymeric substances and the bacterial community in biofilms was affected by both the alloy composition and seawater treatment.

Keywords: steel, alloy, SEM, microbiological corrosion, pitting corrosion.

1. Introduction

Virtually any metal surface exposed to water is susceptible to microbial colonization and biofilm formation. Biofilms are sessile communities of microbial cells and extracellular products associated with a substratum [1-3]. Biofilms seem to be a preferential mode of life for microorganisms as it offers a more sustainable environment for their survival, growth and reproduction [4-6]. In addition, biofilm development allows mutualistic and synergistic interactions between microorganisms and affords protection from external harsh conditions [5, 7]. Within these biofilms, microorganisms can initiate, facilitate or accelerate metal corrosion reactions in a phenomenon known as microbiologically influenced corrosion (MIC) [8-10].

In the construction of subsea pipelines, carbon steel has been the dominant metallic material since it has tremendous advantages of a large experience base and strong technical background along with moderate cost. However, corrosion resistant alloys such as stainless steels and nickel-based alloys are becoming more important structural materials due to their combination of high strength and resistance to corrosion in aggressive offshore environments. Corrosion resistant alloys suffer negligible general corrosion in seawater due to a protective, predominantly chromium oxide, film that forms immediately on the surface with exposure to air [11, 12]. However, these alloys do still suffer localised corrosion in seawater, i.e. pitting corrosion, crevice corrosion, stress corrosion cracking (SCC) and microbiologically influenced corrosion (MIC) [13-16].

Microorganisms do not produce a unique type of corrosion but rather they influence or shift the existing mechanisms for corrosion. Most studies report MIC as a mode of localized corrosion [17-19]. Previous studies on MIC have postulated several mechanisms through which microorganisms can aggravate localized corrosion on active-passive alloys. It has long been known that natural biofilms are able to shift the E_{corr} of active-passive alloys in the noble direction, a phenomenon collectively known as ennoblement. Ennoblement has been widely studied and numerous reports from geographically diverse sites have been published over the years [20-24]. The significance of this phenomenon lies in its influence on the susceptibility to corrosion of anode materials in galvanic couples and the initiation and propagation of localized corrosion [25, 26]. Other MIC mechanisms include biodeposit formation leading to a crevice type of attack [15], acceleration of propagation rates for crevice corrosion and decrease of the critical potentials for pitting and crevice corrosion initiation [14, 27].

The use of natural seawater in the hydrotesting of subsea pipelines prior to commissioning is becoming an issue of increasing importance to the oil and gas industry. This practice can contaminate

the internal surface with microorganisms, sand and salts, even after the water has been removed, increasing the possibility of internal localized corrosion, particularly MIC [28-30]. Therefore, hydrotest seawater must be properly treated in order to reduce the possibility of contamination and severe corrosion damage and to prolong pipeline and equipment service life [31, 32].

Generally, the risk of MIC can be minimized by using chemical biocides to control the growth of suspended cells and further biofilm formation on steels [33, 34]. However, environmental concerns associated with chemical disinfection and biocide disposal have led to the consideration of physical methods for treatment of industrial waters. Furthermore, factors such as chemical incompatibilities, persistence, toxicity, cost and degradation associated with the use of chemical biocides are becoming an issue of increasing importance to the oil and gas industry. Filtration and ultraviolet (UV) light disinfection can be a good supplementary method to traditional chemical treatments for management of industrial waters as it can greatly reduce the amount of chemical biocides required for water disinfection. Filtration and settlement reduce the amount of sediments, larger organisms and non-soluble organic matter which increases the UV light disinfection capacity. UV radiation damages proteins and membranes and indirectly damages DNA by creating reactive oxygen compounds (e.g., H_2O_2 , O_2^- etc.) causing single-strand breaks in DNA which ultimately leads to the death of the microorganisms [35].

In this study, several offshore construction alloys were exposed to slowly flowing natural seawater that ensured a constant loading rate of nutrients and promoted the formation of mature and active biofilms in a test rig established in New South Wales, Australia [36]. The particular design of the test rig allowed for the evaluation of the alloys corrosion performance in raw untreated seawater as well as filtered-UV irradiated seawater. The corrosion performance of the alloys was investigated by monitoring corrosion potential over time and conducting surface analyses. Bacterial diversity in biofilms formed on the different alloys in raw and treated seawater was examined by polymerase chain reaction (PCR) of bacterial 16S rRNA gene fragments followed by denaturing gradient gel electrophoresis (DGGE). Molecular characterization of biofilm communities has become crucial to understand the complexity of the interactions of biofilms with substratum surfaces and the surrounding environment [16, 37-41]. The sensitivity of this technique allowed an assessment of the degree to which exposure conditions and material composition affect the bacterial community and shift the diversity in the biofilms.

2. Experimental details

2.1. Specimen preparation

Commercial carbon steel, stainless steels UNS S31603, UNS S31803, UNS S32750, UNS S31254 and the nickel-base alloys UNS N08825 and UNS N06625 were used in this study. The chemical composition of the alloys in weight per cent is presented in Table 1. Square coupons (20 mm x 20 mm x 5mm thick) were cut from the supplied plate samples and a 2 mm diameter hole was drilled in one corner. An electrical connection was established via a copper wire soldered to one side of the coupon. To prevent crevice corrosion, samples were electrocoated with a protective epoxy (Powercron® 6000CX, PPG Industrial coatings) at the surface area where the spot weld was made for electrical connection and uncovered weld areas further covered by epoxy resin (Belzona 1391, Belzona polymerics Ltd.). Prior to exposure, coupons were wet ground to a 600 grit finish, soaked in Decon® 90 (Decon laboratories Limited) for 3 hours and sterilized by immersion in 70% ethanol for 1 hour. Coupons were finally dried with nitrogen, weighed in triplicate and total coupon areas were measured using a digital gauge. Coupons were suspended by nylon strings in the experimental tanks.

2.2. Test conditions

Two lots of triplicate coupons of each material were exposed to streams of continuous low velocity ($<1 \text{ mm s}^{-1}$) natural coastal seawater in a test rig established in a field laboratory within the Port Stephens Fisheries Centre site at Taylors Beach, New South Wales, Australia. A detailed description of the experimental rig is given elsewhere [36]. One stream was untreated and passed straight into a sealed 200 l experimental tank (referred to as raw seawater). A second stream was pumped into settling tanks followed by a series of filters down to $5 \mu\text{m}$, passed over ultra violet lamps in tandem before going into another sealed 200 L experimental tank. This tank was continuously irradiated with ultraviolet (UV) light (lamp placed above water) in order to kill microorganisms without changing the chemical properties of the water (referred to as treated seawater). The UV lamps used were 40 Watt input UV-C light lamps (254 nm wavelength). UV-C irradiation has been shown to be very effective in controlling marine biofouling development [42]. Lamps were checked daily.

The chemical composition of the seawater is shown in Table 2. Water temperatures in the experimental tanks were recorded daily for the duration of the experiment (Tinytag Aquatic, Gemini Data Loggers, UK).

2.3. Electrochemical studies and determination of corrosion rates

The corrosion potential (E_{corr}) of each electrode was measured against a type CCS1-PORT Ag/AgCl portable seawater reference electrode (Silvion Limited, accuracy Vs SCE in 3% NaCl at 20°C: -5mV +/-5mV) and recorded every four hours using a multichannel data logger (dataTaker DT605, dataTaker Pty Ltd). Coupons were withdrawn after 90 days immersion, cleaned following the standard procedure [43] and weighed in triplicate to calculate weight loss and corrosion rates of each sample [43]. The average corrosion rate over the 3 month exposure is calculated according to the following equation (Eq. (1)):

$$\bar{r} = k\bar{w} / \rho A t, \quad (1)$$

where \bar{r} = corrosion rate, mm y⁻¹; k = constant, 8.76 x 10⁴; \bar{w} = weight loss, g; ρ = density, g cm⁻³; A = area in cm²; t = time of exposure in h.

In calculating an average, it is not specifically implied that the corrosion rate remains constant for this time. Neither is it implied that this corrosion rate will be sustained for periods of years, but rather the measured corrosion is presented in units of mm y⁻¹ for the purpose of comparison with well recognized values in this unit. One-way analysis of variance (ANOVA) was conducted to examine the significance of variations in corrosion rates between treated and untreated seawater coupons of the same type at a significance level of 0.05 (calculated as statistical p values).

2.4. Evaluation of biofilm formation by SEM

Natural marine biofilms formed on the coupons exposed to coastal seawater under freely corroding conditions were examined by scanning electron microscopy (SEM). After 90 days exposure, biofilm-coated coupons were removed from experimental tanks and fixed in glutaraldehyde (2.5% in 0.025 M phosphate buffered saline (PBS) (Sigma), pH 7.4) containing 0.15% w/v Alcian Blue (Sigma Aldrich) and incubated at room temperature for a minimum of 22 h. Coupons were then washed with PBS (0.025 M, pH 7.4) and fixed in sterile 1% osmium tetroxide for 30 min. Coupons were then dehydrated through a graded series of ethanol (15 min each): 50, 75, 90 and 100% dry ethanol successively. Dehydrated coupons were critical-point dried (CPD) in liquid carbon dioxide using an E3000 Critical Point Dryer (Quorum Technologies). After CPD, coupons were sputter-coated with

platinum (5 nm thickness) using a SC7640 Sputter Coater, Quorum Technologies. SEM imaging was performed on a Zeiss 1555 VP-FESEM using an in-lens detector with a 30 mm aperture, accelerating voltage of 3 kV and a working distance of 4 to 5 mm.

2.5. Optical surface analysis for corrosion evaluation

Localized corrosion of the alloys was evaluated by surface optical measurements using an infinite focus microscope (IFM G4g system, Alicona Imaging). One-way analysis of variance (ANOVA) was conducted to examine the significance of variations in pit depths and pit density analyses between treated and untreated seawater coupons of the same type at a significance level of 0.05 (calculated as statistical p values).

2.6. Analysis of bacterial diversity by PCR-DGGE

The diversity in the biofilm communities developed on the test alloys exposed to raw and treated seawater was examined by PCR-DGGE analysis. Applying this method, different DNA sequences from individual bacterial species in a biofilm community can be separated based on differences in their melting behavior on a denaturing gradient gel [41]. By comparing the melting behavior, or the positioning of the DNA bands, side-by-side on the gel, it is possible to detect differences in biofilm community composition and diversity.

At the completion of the 90 days exposure period, duplicate coupons of each material were removed from the experimental tanks and resuspended separately in small vials containing sterile seawater and 0.1 % (w/v) Tween 20. Microbial cells were detached by 60 second-sonication steps (solution was refreshed between sonication steps) until no microbial cells were observed in the solution under a phase contrast microscope. For each material, suspensions from all sonication steps were filtered through 0.2 μ m pore diameter polycarbonate membrane filters (Isopore™, Millipore Corp.) and DNA from microorganisms on the membrane filters was extracted using a DNA extraction kit (PowerSoil™ DNA Isolation Kit, MO BIO Laboratories Inc). DNA was used as template to amplify a specific region of the bacterial 16S rRNA gene using nested PCR approach. The outer primer pair was 27F and 1492R and the inner primer pair was 357F-GC and 907R (Table 3) [44, 45]. The PCR-amplified 16S rRNA gene fragments from different species were separated by DGGE using a DCode Universal Mutation Detection System (BIORAD) as per the manufacturer's instructions. The PCR products were mixed with DNA loading buffer (BIOLINE) and loaded into 7% (w/v) polyacrylamide gel (40% acrylamide/bis solution, 37.5:1) with a denaturing gradient of 30-60% urea-formamide (100% denaturant: 7M urea, 40% deionised formamide) in 1x TAE buffer (2 M Tris base, 1 M glacial acetic acid, 50 mM, EDTA, pH 8.0). The DGGE was run at 150 V at 60°C for 16 hours. DNA bands

were visualized using a gel illuminator following staining with SYBR® Gold nucleic acid gel stain (Invitrogen™).

More detailed analyses of individual biofilm populations, such as DNA sequencing and speciation, are beyond the scope of the present study.

3. Results

3.1. Corrosion Potential

Figure 1 shows average E_{corr} evolution in time of the test materials exposed to raw and treated seawater for 90 days. For all the corrosion resistant alloys exposed to raw seawater (Figure 1 [a-f]) E_{corr} shifted from negative to positive values during the first 5 days of exposure. For most of the alloys, E_{corr} continued to increase with time and only minor active-passive states were observed over the exposure period. For UNS S31603 (Figure 1[a]), large active-passive peaks were more evident during exposure as compared with the other alloys. For most of the alloys, these active peaks were detected after 20-30 days exposure to raw seawater except for UNS N08825 (Figure 1[f]) where a drastic shift of E_{corr} towards active values was observed after a maximum ennoblement had been attained during the first 5 days of exposure. E_{corr} of all alloys exhibited a clear tendency to enoble with exposure. Corrosion resistant alloys exposed to filtered-UV treated seawater displayed a fairly stable E_{corr} during the first 40 days of exposure with a slight trend towards negative values with time. It can be seen that after 40 days exposure, E_{corr} of all alloys exposed to treated seawater shifted to electropositive values and increased gradually with exposure. After this initial ennoblement, E_{corr} of some of the alloys exposed to treated seawater displayed active-passive transition peaks. In all alloys, E_{corr} ennobled approximately +400-500 mV relative to the initial E_{corr} . The maximum ennoblement attained during exposure was slightly higher for alloys exposed to raw seawater compared to alloys exposed to treated seawater although values were comparable. E_{corr} of carbon steel exposed to raw seawater (Figure 1[g]) slightly decreased during the first 5 days exposure and then displayed a smooth tendency towards more positive values with time. E_{corr} of carbon steel was ennobled about +50 mV relative to the initial E_{corr} . E_{corr} of carbon steel exposed to treated seawater also showed a slight increase over time although the material did not show considerable ennoblement.

3.2. Temperature

The exposure temperature in the experimental tanks measured over the course of the immersion period is plotted in Figure 2. Initial exposure temperatures were about 18-19°C and reached maximum values of ~28°C after 30 days of exposure. Average temperatures were 23.1°C and 22.7°C for treated and raw seawater tanks, respectively. The slight increase in temperatures in the treated seawater tank is attributed to the effect of UV lamps.

3.3. Corrosion rates

Average corrosion rates calculated from weight loss of test materials are shown in Figure 3. Corrosion rates of high corrosion resistant alloys (Figure 3[a]) were very low in both in raw and treated seawater (displayed in $\mu\text{m y}^{-1}$). UNS N08825 exhibited the highest corrosion rates between the high corrosion resistant alloys in both raw and treated seawater ($\sim 0.3 \mu\text{m y}^{-1}$) and UNS N06625 showed the lowest corrosion rates between these alloys ($\sim 0.05 \mu\text{m y}^{-1}$).

Carbon steel (Figure 3[b]) displayed corrosion rates of $\sim 0.12 \text{ mm y}^{-1}$. There was not significant variation in the corrosion rate of alloys exposed to treated and untreated seawater (UNS S31603: $p = 0.37$; UNS S31803: $p = 0.40$; UNS S32750: $p = 0.40$; UNS S31254 $p = 0.39$; UNS N6625: $p = 0.10$; UNS N8825: $p = 0.52$; Carbon steel: $p = 0.28$).

3.4. Surface analysis

Surface analysis by optical microscopy was conducted at the completion of the exposure to evaluate localized corrosion. Carbon steel showed severe localized corrosion (Figure 4) and the extent of the attack was higher in the raw seawater than in the treated seawater. For carbon steel, pit densities could not be measured due to the nature of the attack. Carbon steel exposed to raw seawater was severely attacked on the corners (Figure 4[a]) whilst random localized attack throughout the surface was observed in carbon steel exposed to treated seawater (Figure 4[b]). Carbon steel exposed to raw seawater exhibited significantly higher maximum depths of attack ($500 \mu\text{m}$ at the corners) than carbon steel in treated seawater ($50 \mu\text{m}$) ($p < 0.05$).

Figure 5 shows the pitting analysis (pit density and pit depths) for the corrosion resistant alloys studied. Pitting corrosion was observed on the alloys UNS S31603 and UNS N08825 exposed to raw and treated seawater and was not detected on UNS S32750, UNS S31254 and UNS N06625. UNS N08825 exhibited the highest pit density as well as the highest average and maximum pit depths in both raw and treated seawater. A few shallow pits (less than $10 \mu\text{m}$ depth) were found on UNS S31803 exposed to both raw and treated seawater. UNS S31603 and UNS N08825 exposed to raw

untreated seawater exhibited significantly higher pit density and maximum pit depths than treated seawater coupons ($p = < 0.05$). In contrast, there was not significant variation in pit density ($p = 0.22$) and maximum pit depths ($p = 0.13$) between UNS S31803 exposed to treated seawater and UNS S31803 in raw untreated seawater.

Grain structures were evident on a small region of the surface of UNS S31603 exposed to raw seawater and intergranular corrosion (IGC) was apparent (Figure 6). No evidence of intergranular corrosion (IGC) was found on UNS S31603 exposed to filtered-UV treated seawater.

3.5. Biofilm imaging

SEM images of biofilms developed on the test alloys after 90 days exposure to seawater are shown in Figure 7. Biofilms were observed on coupons exposed to raw seawater as well as treated seawater. From SEM imaging, there were no noticeable differences in physical structure between biofilms formed in raw and treated seawater. However, the pattern of colonization and biofilm structure differed between materials. Ennobling biofilms on corrosion resistant alloys consisted of microbial cells, diatoms and extracellular polymeric substance (EPS) in both treated and raw seawater (Figure 7[a]). However, diatoms were much less abundant in biofilms formed on alloys exposed to treated seawater than raw seawater.

Biofilms on UNS S31603 (high pit density) were thin and irregular along the surface forming net-like structures and patchy deposits and aggregates (Figure 7[b]). The alloy surface was not fully covered by biofilms. Biofilms formed on UNS S31803 (shallow pits), UNS S32750 and UNS S31254 (not pitted) partially covered the surface and were thicker and more complex than those formed on UNS S31603 (Figure 7[c]). Biofilms developed on the nickel based alloys UNS N08825 (high pit density and pit depths) and UNS N06625 (not pitted) were indistinguishable between the two alloys. These biofilms were very dense with microbial cells, filamentous structures and diatoms embedded in copious amounts of EPS and fine overlapping material on the entire surface (Figure 7[d]). This fusing material appeared as a connecting structure among biofilm components. These biofilms were most often aggregated into high and irregular structures. On the other hand, carbon steel surfaces show individual microbial cells and aggregates encrusted with corrosion products over the entire surface in both treated and raw seawater tests (Figure 7[e]). There was no correlation found between biofilm thickness and the extent of pitting corrosion on the different materials.

3.6. PCR-DGGE analysis of bacterial diversity

PCR-DGGE analysis of biofilms revealed a shift in bacterial community in biofilms as a function of substratum material (Figure 8). Bacterial DNA was extracted from all the materials exposed to both treated and raw seawater. The individual bands seen in each lane of the DGGE are related to the

predominant bacterial populations present in each biofilm formed on the different materials and the number of bands gives an indication of the bacterial diversity in biofilms. DGGE analysis indicated that for each type of material the number and types (indicated by the melting behaviour and positioning of the band in the gel) of bacterial populations in biofilms formed in raw seawater differed from those formed in treated seawater (encircled bands for each material in Figure 8). However, for each material certain bacterial species were part of both the biofilm community developed on the raw seawater and the one developed on the treated seawater (DNA bands from raw and treated seawater biofilms showing the same positioning on the gel). Likewise, several bacterial species were commonly encountered in biofilms developed on different materials although some species were only found in biofilms grown on certain materials but not on others. No obvious decrease in DGGE band numbers in the treated seawater was observed compared to the raw seawater. UNS N08825, UNS S31603 and UNS S31803 exhibited the highest microbial diversity (8 to 10 bands) between the different materials.

4. Discussion

The use of preservation treatments for industrial waters is a crucial practice to provide protection of subsea pipelines against MIC. Issues surrounding the use of chemical biocides to control biofilm growth on internal surfaces of offshore structural alloys have led to the interest in alternative seawater treatments for mitigating biofilm growth and preventing MIC. In this work, the influence of filtration-UV irradiation on the control of biofilm growth and activity on several alloys and finally on MIC in seawater was investigated. As it has been demonstrated in previous studies, results from this work indicate that microorganisms can rapidly colonize the steel surfaces immersed in natural seawater and enoble their E_{corr} . The extent of the ennoblement observed in these experiments was similar to values reported by researchers in other geographical regions [20, 46, 47]. Previous studies on the phenomenon of ennoblement have indicated that the rapid ennoblement of E_{corr} of steels in natural waters is directly associated with the formation of a biofilm on the surface following exposure. Hence, it can be inferred from the present study that filtration-UV radiation was effective in delaying biofilm formation and consequently ennoblement of the alloys corrosion potential for up to 40 days. The drastic E_{corr} ennoblement observed simultaneously on all test alloys after the 40-day exposure to treated seawater could be due to a temporary failure of the UV system likely during off-working hours when the test rig can be unattended. Under these conditions, new seawater microorganisms introduced into the experimental tank can colonize the alloys surface within a few hours of exposure and rapidly form a biofilm matrix. Once a biofilm is established, microbial cells can be sheltered by exopolymeric substances, allowing an apparent increased UV resistance [48]. The above assumption is consistent with the persistence of the ennoblement observed on the alloys exposed to the UV treated seawater from the day 40 until the end of the exposure period. The biofilm formation on alloys exposed to both

treated and raw seawater was confirmed by SEM imaging. However, since time-course studies of biofilm formation were not performed (only a single-point measurement was conducted at the end of exposure) it is not possible to determine when exactly a biofilm was formed on the alloys surface. Nevertheless, It is commonly reported that initiation of ennoblement is directly related to microbial colonization and biofilm growth on the steel surface [49].

Several mechanisms have been proposed to explain the phenomenon of ennoblement but unifying mechanisms for global observations have not been identified. Numerous researchers have shown that increased cathodic reduction rates accompany ennoblement of E_{corr} [20, 50]. In marine waters, this has been attributed to organometallic catalysis [51], acidification of the metal surface [52], catalysis by bacterially produced enzymes [23, 24], the combined effects of low pH and production of hydrogen peroxide within biofilms [52] and microbially produced inhibitors [53]. More recently, it has been demonstrated that certain bacteria are able to switch from natural soluble electron acceptors and donors to solid electrodes so that there is a direct transfer of electrons between bacterial cells and conductive surfaces. Direct microbial electron extraction from solid electrodes has shown to be induced by carbon starvation [19]. In the absence of a soluble organic electron donor some bacteria can develop electron transfer capability where the solid electrode serves as the only electron donor to support growth without the need for soluble electron mediators. Under these conditions bacteria have shown to catalyse the cathodic reactions and enoble the electrode potential [54, 55]. This direct electron uptake mechanism seems to be mediated by membrane bound electron proteins such as c-type cytochromes and bacterial nanowires that transfer electrons from outside the cells to the cytoplasm and vice versa. The enhanced cathodic reaction by microbial direct electron transport is thought to be less sensitive to mass-transfer compared to reactions using oxygen or other soluble species. This is because mass transfer resistance within biofilms can limit the diffusion of soluble cathodic reactants from the bulk solution to the metal surface thus hindering the corrosion reaction.

Ennoblement has also been related to the settlement of living sea diatoms on stainless steels [20]. It was suggested that ennoblement was caused not by the metabolism but by the metabolite of the sea diatoms contained in a thin organic film adhered to the steel. In the present study, sea diatoms were found embedded in the EPS of ennobling biofilms on the different alloys which may suggest they could have played a role in the observed ennoblement. It is probable that all of these mechanisms, either singly or in combination, may contribute to the ennoblement of the alloys. However, the precise mechanisms of ennoblement by biofilms on the selected corrosion resistant alloys were not investigated in this work.

Surface analysis indicated that localized corrosion occurred on carbon steel and on the corrosion resistant alloys UNS S31603, UNS N08825 and UNS S31803 exposed to both raw and treated seawater. Localized corrosion was not detected on UNS S32750, UNS S31254 and UNS N06625

despite the large ennoblement of E_{corr} attained by these alloys during exposure. Similarly, no correlation was found between E_{corr} values and the uniform corrosion rates observed on all selected alloys in seawater. Corrosion rates by weight loss of corrosion resistant alloys in seawater did not give a direct indication of their pitting corrosion susceptibility in seawater. These findings highlight that for corrosion assessment, weight loss measurements are only appropriate for indicating the uniform corrosion tendency of alloys at particular environments. The results are difficult to interpret in the presence of localized corrosion such as pitting, the most frequent form of attack found in passive alloys in chloride environments. Under these conditions, electrochemical analysis of localized corrosion as well as pitting analysis from surface inspection such as pit density, pit depth and pit volume offer a more accurate indication of the pitting tendency of passive alloys in seawater.

Results from this study can be explained on the basis of the known potential-temperature dependence on the onset of localized corrosion in active-passive alloys. It is known that localized corrosion can take place if the corrosion potential of steel in a given environment surpasses a critical potential provided a critical temperature is exceeded [13, 56, 57]. The critical potential is usually considered to be the crevice corrosion repassivation potential (E_r). At lower potentials than E_r , pit initiation is followed by rapid repassivation, a stage commonly referred to as metastable pitting. Both a critical potential and temperature are required to stabilize pit growth on the alloys surface. In a previous study, critical potentials and critical temperatures for pitting and crevice corrosion of high-resistance alloys in seawater were investigated [13]. It was observed that critical potentials for pitting and crevice corrosion initiation and repassivation decreased with increasing exposure temperature and lowering of temperature similarly ennobled the critical potential. In the same study, UNS S32750 and UNS S31254 exhibited excellent resistance to crevice corrosion in natural seawater at temperatures below 40°C under potentiostatic and potentiodynamic test conditions. This is in agreement with the results from the present study where UNS S32750, UNS S31254 and UNS N06625 remained protected against localized corrosion regardless of the presence and activity of a biofilm on their surfaces. On the other hand, UNS S31603, UNS S31803 and UNS N08825 seem to be prone to initiate localized corrosion at the seawater temperatures attained in our test rig as indicated by previous studies on their crevice corrosion repassivation potentials in seawater [13].

It is important to point out that results from previous investigations on the resistance of the alloys to localized corrosion in seawater were obtained using freshly ground coupons. In the presence of biofilms, a more aggressive microenvironment is created at the biofilm-metallic interphase which can facilitate localized corrosion initiation processes. Generally, these microfouling deposits present a patchy distribution on the metal surface so that the physical presence of a biofilm acts as a diffusion barrier that retards the movement of species from the bulk solution toward the metal surface and from the metal-biofilm interface outwards into the solution. Under these conditions, repassivation can be

inhibited by limiting oxygen diffusion to the metallic surface leading to pit stabilization by differential aeration cell. In fact, biofilms have been reported to decrease initiation times for localized corrosion by lowering the critical initiation and repassivation potential for pitting and crevice corrosion [14, 16]. It is very likely that the above mechanisms in conjunction with the observed ennoblement of the E_{corr} compromised the stability of the passive film and assisted localized corrosion initiation provided the exposure temperature had exceeded a critical value. Moreover, ennoblement of E_{corr} decreases the critical temperature below which a given alloy should be resistant to localized corrosion initiation.

Surface analysis also indicated that UNS S31603 suffered intergranular corrosion (IGC) only when exposed to raw untreated seawater. IGC is a form of localized corrosion along grain boundaries caused by anodic dissolution of specific regions at the surface such as regions depleted of alloying elements. Non-uniform intergranular attack can be due to factors that locally impair the corrosion process such as the formation of deposits. This may weaken the oxide film at specific locations, allowing halides such as chloride ions greater access to the underlying metal and facilitating localized attack. IGC has been previously reported for 70Cu-30Ni alloy under elliptical deposits of embedded diatoms [58] whereas IGC was not observed on the same alloy exposed to artificial seawater. It was suggested that dissolved sulphides produced by bacteria may have reacted with nickel in grain boundaries causing preferential dissolution of the steel in these regions. It has been shown that selective colonization by bacteria results in depletion of Cr and Fe relative to nickel at the grain boundaries on 316L stainless steel [59]. It was also reported that individual cells were seen along surface grain boundaries prior to biofilm formation suggesting that initial colonization is non-random and highly selective for grain areas. In addition, it was shown that sulphur compounds preferentially accumulate at grain boundaries particularly in the presence of sulphate-reducing bacteria (SRB). On the basis of these findings, it is reasonable to consider that microorganisms played a role in the onset of IGC of UNS S31603 exposed to raw seawater. However, since biofilms also formed on UNS S31603 exposed to UV treated seawater, where IGC was not detected, IGC cannot be attributed to the mere presence of microorganisms. Given that PCR-DGGE analysis revealed differences in the bacterial community in biofilms formed in raw and UV treated seawater, it is possible that IGC initiation could have been particularly favoured by the distinctive metabolic activities of the biofilm community developed in the raw seawater system. It is plausible to expect that microbial activity and biomass generation was higher in raw untreated seawater, where nutrients and organic matter are more abundant compared to the filtered-UV irradiated seawater.

PCR-DGGE method was used for directly determining the genetic diversity of microbial populations in the complex biofilms developed on the test alloys. PCR-DGGE analysis of biofilms formed on alloys exposed to filtered-UV treated seawater as well as raw untreated seawater indicated that filtration-UV irradiation had an effect on biofilm community composition. Changes in bacterial

community in biofilms developed in treated seawater were likely associated with lower concentration of nutrients and organic matter due to filtration and settlement. PCR-DGGE also revealed that bacteria differed in their preference for material type. Different biofilm communities on different alloys have been reported previously [14]. It is likely that microbial adhesion and biofilm formation are affected by the nature of the passive film and the microstructure of alloys. Chemical composition and microstructure have been shown to have a strong influence on the composition and thickness of oxide films, which may contribute to the selective attachment of microorganisms.

Interestingly, a higher bacterial diversity was observed on alloys that exhibited some degree of pitting corrosion (UNS N08825, UNS S31603 and UNS S31803) compared to the alloys that displayed resistance to pitting. It is difficult, however, to discriminate between microbiological events that influenced pitting corrosion and corrosion reactions that influenced microbial settlement and diversity. The spatial distribution of bacteria on corroding surfaces has been previously investigated [60]. Autoradiography of ^{14}C -acetate labelled bacteria on well-established anodic regions demonstrated that bacteria preferentially bound to sites of active anodic activity such as corrosion products formed over corrosion pits and inactive pits that has initiated and re-passivated. It has been suggested that active corroding sites may offer alternative energy sources for bacterial growth on steel surfaces [61]. These studies, however, only measured bacterial density and did not evaluate bacterial diversity. Nonetheless, previous studies have found no correlation between bacterial diversity and localized corrosion in seawater [16, 37]. From the present data it is difficult to infer any direct association between bacterial diversity in biofilms and localized corrosion events.

In addition, SEM revealed variations in physical structure and amount of EPS produced in biofilms according to substratum material. Differences in EPS production could be a bacterial mechanism to promote hydrophobic interactions to favour irreversible sorption onto solid surfaces [62]. The higher amount of EPS observed on nickel-based alloys compared to stainless steels, could suggest that there is a lower affinity between nickel oxide films and microbial cells compared to oxides formed on stainless steels. Likewise, the presence of toxic metals in the environment has been shown to stimulate EPS production by microorganisms [63] and the toxicity of nickel towards microorganisms is well documented [64]. Based on these reports, our findings could also suggest that EPS production was increased as a bacterial defence mechanism against toxic nickel species from corroding nickel-based alloys.

Results from this investigation highlight the impact of filtration, settlement and UV irradiation on corrosion performance and MIC in seawater. Although filtration-UV irradiation did not guarantee sterilization of the water and did not eradicate established biofilms it proved to be an efficient approach for mitigating biofilm growth and activity on corrosion resistant alloys in seawater. Filtration and settlement reduce the levels of nutrients and carbon sources in seawater which affects bacterial

diversity in biofilms and limits biofilm growth and activity on alloys. In addition, filtration can reduce the number of biofouling organisms such as diatoms which are known to contribute to the corrosion of the steels. The use of filtration-UV irradiation to mitigate biofilm growth-associated effects on corrosion resistant alloys can provide an environmentally friendly practice to prevent MIC of subsea equipment. The appropriate use of this approach could contribute to reducing the overdosing of chemical biocides thus potentially decreasing the costs and impacts associated with their discharge to the environment. The technique however, is not effective against biofilms and hence should not be considered alone but rather in conjunction with other microbial control methods to fully protect subsea equipment against MIC.

5. Conclusions

Filtration-UV irradiation has promise as a microbial control method to prevent MIC of offshore construction alloys. Natural marine biofilms ennobled E_{corr} of the stainless steels UNS S31603, UNS S31803, UNS S32750, UNS S31254 and the nickel alloys UNS N08825 and UNS N06625 by as much as 400-500 mV relative to their initial E_{corr} (Ag/AgCl) within a few days of exposure to raw natural seawater. Treatment of seawater using filtration UV-C irradiation delayed microbial electrochemical activity on the alloy surfaces for up to 40 days which resulted in lower uniform and localized corrosion rates. Likewise, PCR-DGGE indicated that seawater treatment has an impact on biofilm community composition most likely due to the shortage of nutrients, organic matter and other colonizing organisms resulting from filtration and settlement.

Localized corrosion was only observed on carbon steel, UNS S31603 and, UNS N08825 and UNS S31803 and not detected on UNS S32750, UNS S31254 and UNS N06625 regardless of the presence of biofilms, most likely due to the high potential and temperature dependence of these alloys to initiate localized corrosion in natural seawater. Localized corrosion, as determined by pit depth and pit density analyses, was significantly higher in carbon steel, UNS N08825, UNS S31603 exposed to raw untreated seawater compared to exposures in treated seawater. UNS S31803 showed the lowest pitting corrosion between susceptible alloys with no significant differences between treated and untreated coupons.

Biofilm community composition differed on the various alloys which can be related to the nature of the passive films and the microstructure on the different alloys. In addition, SEM analysis showed differences in physical structure and EPS production in biofilms according to the type of material. On carbon steel, individual microbial cells were encrusted with corrosion products over the entire surface. Biofilms formed on nickel alloys appeared more abundant, complex and exhibited more copious amounts of EPS compared to the stainless steels. Despite the differences in biofilm morphology, structure and composition on the different alloys the extent of ennoblement was comparable between

the alloys. Filtration-UV irradiation alone was not effective against biofilms in the long term and additional countermeasures would be necessary to fully protect a system against MIC.

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Figures

Figure 1. E_{corr} as a function of time for test materials exposed to raw seawater and filtered-UV irradiated (treated) seawater. [a] UNS S31603; [b] UNS S31803; [c] UNS S32750; [d] UNS S31254; [e] UNS N06625; [f] UNS N08825 and [g] carbon steel.

Figure 2. Variation of temperature over time for flowing raw and treated seawater in the experiments.

Figure 3. Corrosion rates by weight loss of test materials exposed to flowing natural coastal seawater for 90 days. [a] high-resistance alloys in raw and treated seawater (corrosion rates given in $\mu\text{m y}^{-1}$) and [b] carbon steel in raw and treated seawater (corrosion rates given in mm y^{-1}). Errors bars indicate standard deviations from triplicate samples.

Figure 4. 3D optical microscope surface images of carbon steel exposed to flowing natural coastal seawater under open-circuit conditions after biofilm removal and surface cleaning. [a] Localized corrosion on coupons exposed to raw seawater. Localized attack was more severe on the coupon edges with maximum depth of attack of $500 \mu\text{m}$; [b] Severe pitting on coupon exposed to treated seawater; maximum depth of attack of $50 \mu\text{m}$. Coupon edges were not affected.

Figure 5. Surface analysis of UNS S31603, UNS S31803 and UNS N08825 exposed to flowing raw and treated seawater. [a] average pit density (pits cm^{-2}); errors bars indicate standard deviations from duplicate samples and [b] average pit depth (μm); errors bars show the range of pit depths found for each alloy.

Figure 6. 3D optical surface image showing intergranular corrosion (IGC) on UNS S31603 exposed to raw seawater. IGC was not observed on UNS S31603 exposed to treated seawater.

Figure 7. SEM micrographs of biofilms formed on the various test materials exposed to flowing coastal seawater. [a] biofilm containing microbial cells and diatoms (indicated by arrows) embedded in extracellular polymeric substance (EPS) on UNS S31803 exposed to raw seawater; [b] biofilms consisting of mats of fused overlapping material and net-like structures formed on UNS S31603 exposed to treated seawater; [c] mature biofilm showing microbial cells embedded in abundant EPS on UNS S32750 exposed to treated seawater; [d] dense and complex biofilm formed showing diatoms and microbial cells, filamentous structures and diatoms embedded in copious amounts of EPS with connecting structures formed on UNS N08825 exposed to raw seawater; [e] microbial cells encrusted with corrosion products over the surface of carbon steel coupons exposed to raw seawater. A close-up

of the same surface highlights an individual microbial cell (pointed out by a white arrow) on corrosion products.

Figure 8. PCR-DGGE profiles of dominant bacterial populations in biofilms formed on the different test materials exposed to both raw and treated seawater. Each lane of the gel corresponds to one biofilm community and each band theoretically corresponds to one bacterial population in each biofilm community. Red circles highlight at least one of the distinctive bands (populations) that were unique to the raw or the UV treated seawater biofilm for each test material.

Highlights

- > Biofilms ennobled E_{corr} of offshore construction alloys in natural seawater.
- > Filtration-UV irradiation delayed biofilm growth and activity on alloys.
- > Localized corrosion in seawater was lowered by the use of filtration-UV irradiation.
- > Biofilm community composition was affected by both substratum and seawater treatment.
- > Filtration-UV irradiation can be an ecofriendly practice for protection against MIC.

Table 1. Test materials and their chemical composition.

Material	Type	C	Mn	Fe	Cr	Ni	Mo	N	Nb	S
		wt %	wt %	wt %	wt %	wt %	wt %	wt %	wt %	wt %
UNS S31603	Austenitic SS	0.022	1.76	bal	17.4	10	2.03	0.046	-	0.001
UNS S31803	Duplex SS	0.015	1.53	bal	22.35	5.72	3.16	0.18	-	0.001
UNS S32750	Super Duplex SS	0.019	0.819	bal	24.74	6.61	3.73	0.262	-	0.0003
UNS S31254	Super austenitic SS	0.01	-	bal	20.18	18.15	6.1	0.2	-	0.010
UNS N08825	Nickel base alloy	0.05	0.85	22	22.5	bal	3	-	-	0.03
UNS N06625	Nickel base alloy	0.1	0.45	5	22.5	bal	9	-	3.85	0.015
Carbon steel	ASTM A572-50	C 0.155, Al 0.025, Mn 0.65, P 0.020, S 0.010, Si 0.15.								

Table 2. Characteristics of the coastal seawater at the experimental rig (Taylors Beach, NSW, Australia) during the period of this study.

Analysis	Raw seawater	Treated seawater
Ammonia as N [mg l ⁻¹]	0.0085	0.051
Dissolved Oxygen [% saturation]	86.7	82.1
Nitrate as N [mg l ⁻¹]	0.044	0.086
Nitrite as N [mg l ⁻¹]	0.003	<0.002
Salinity as Cl [g l ⁻¹]	34.2	34.3
Sulphate as S [mg l ⁻¹]	3500	3300
Total Phosphorous as P [mg l ⁻¹]	0.015	0.028

Table 3. Primers used in this study for the amplification of the full-length and variable region of the bacterial 16S rRNA gene.

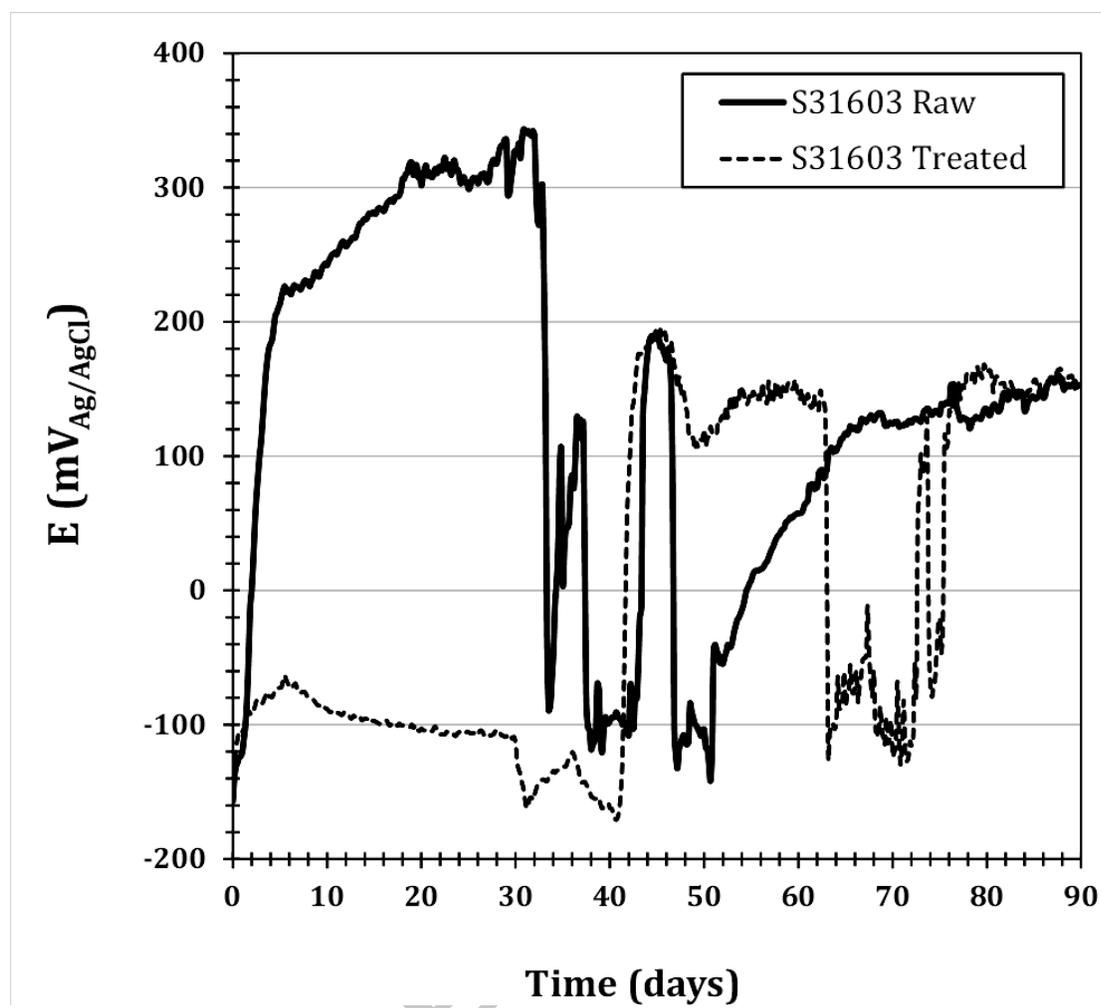
Primer ^a	Amplification target	Sequence (5' to 3')
27F ^b	Bacteria	GAGTTTGATCCTGGCTCAG
357F	Bacteria	CCTACGGGAGGCAGCAG
357F-GC ^c	Bacteria	CCTACGGGAGGCAGCAG
1492R ^{b,d}	Prokaryotes	ACGGdITACCTTGTTACGACTT
907R ^b	Universal	CCG TCA ATT CMT TTG AGT T

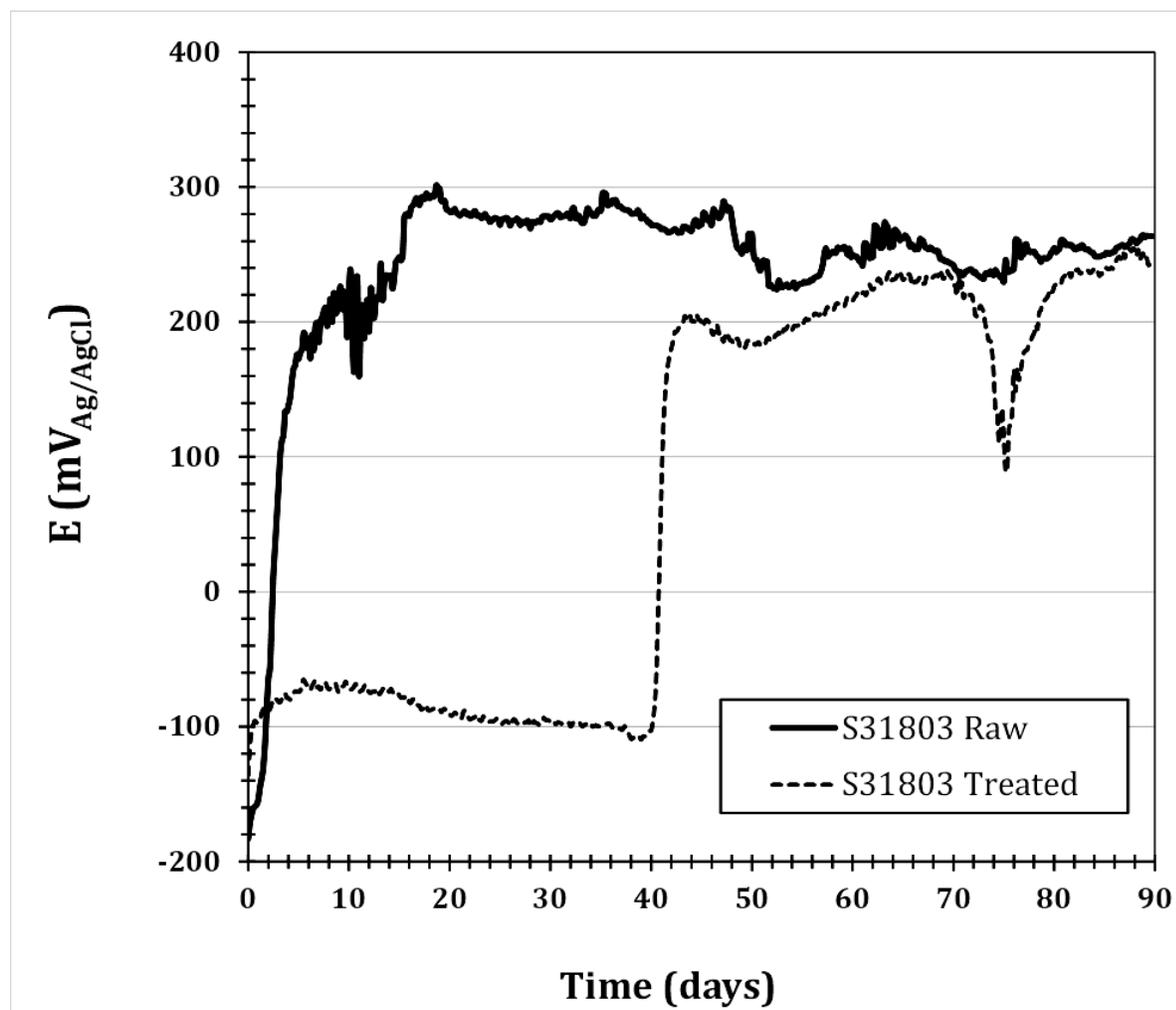
^aThe number relates to the *Escherichia coli* position to which the 3' end of the primers anneals. F and R correspond to forward and reverse primers, respectively.

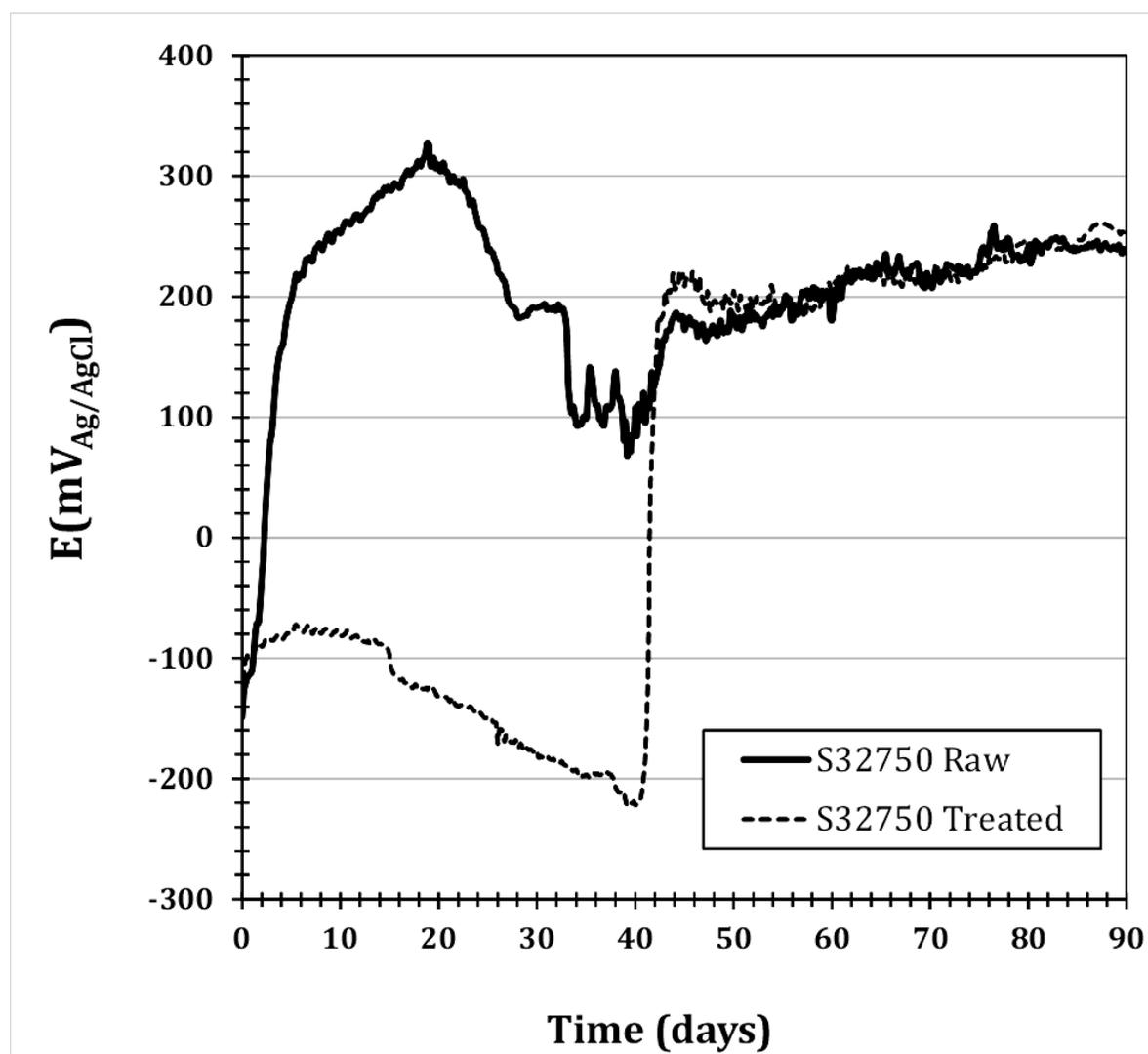
^bModified from the original paper

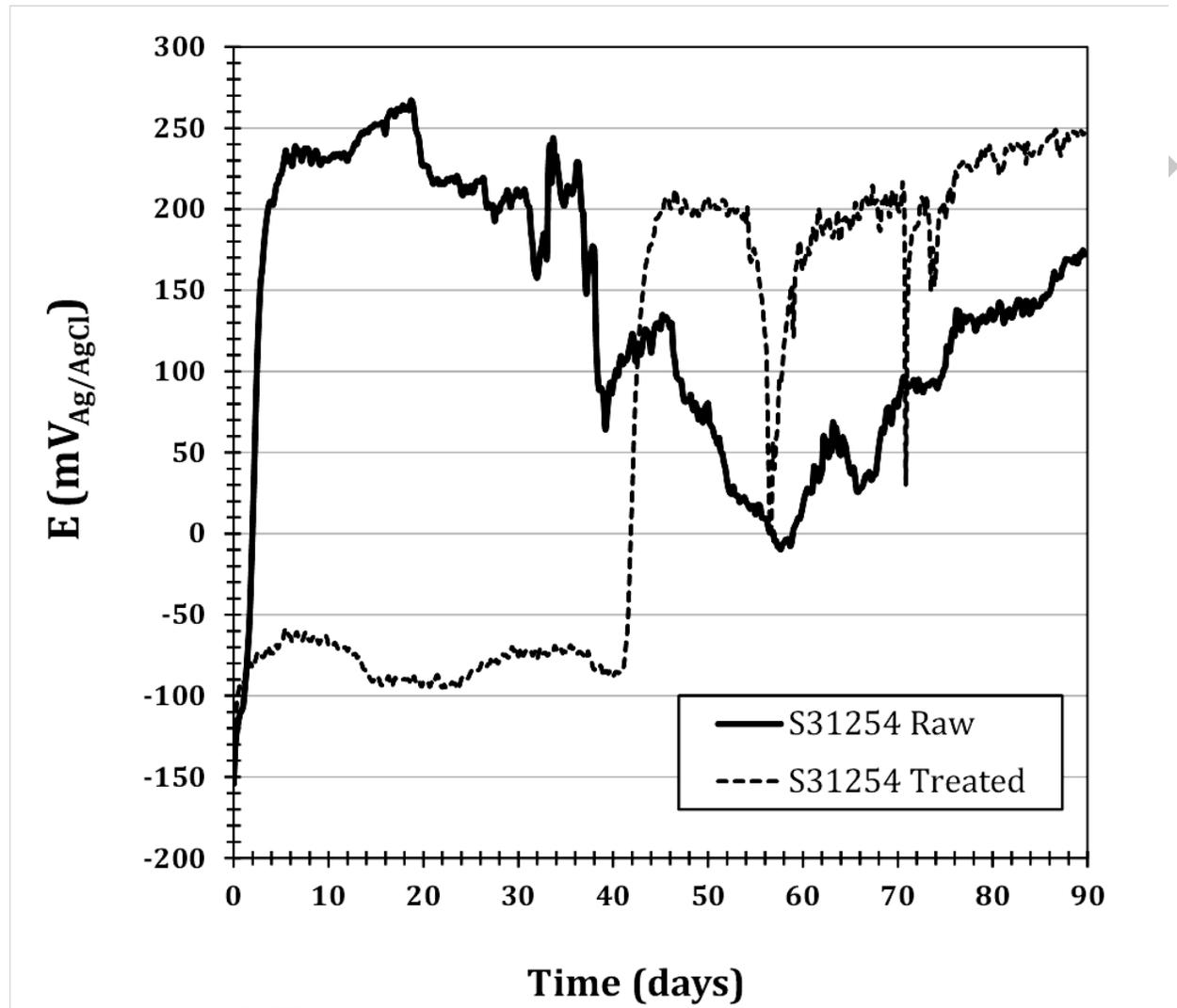
^cGC is a 40 nucleotide GC-rich sequences attached to the 5' end of the primer. The GC sequence is 5'-CGCCC GCCGCGCGGGCGGGCGGGGGCACGGGGGG.

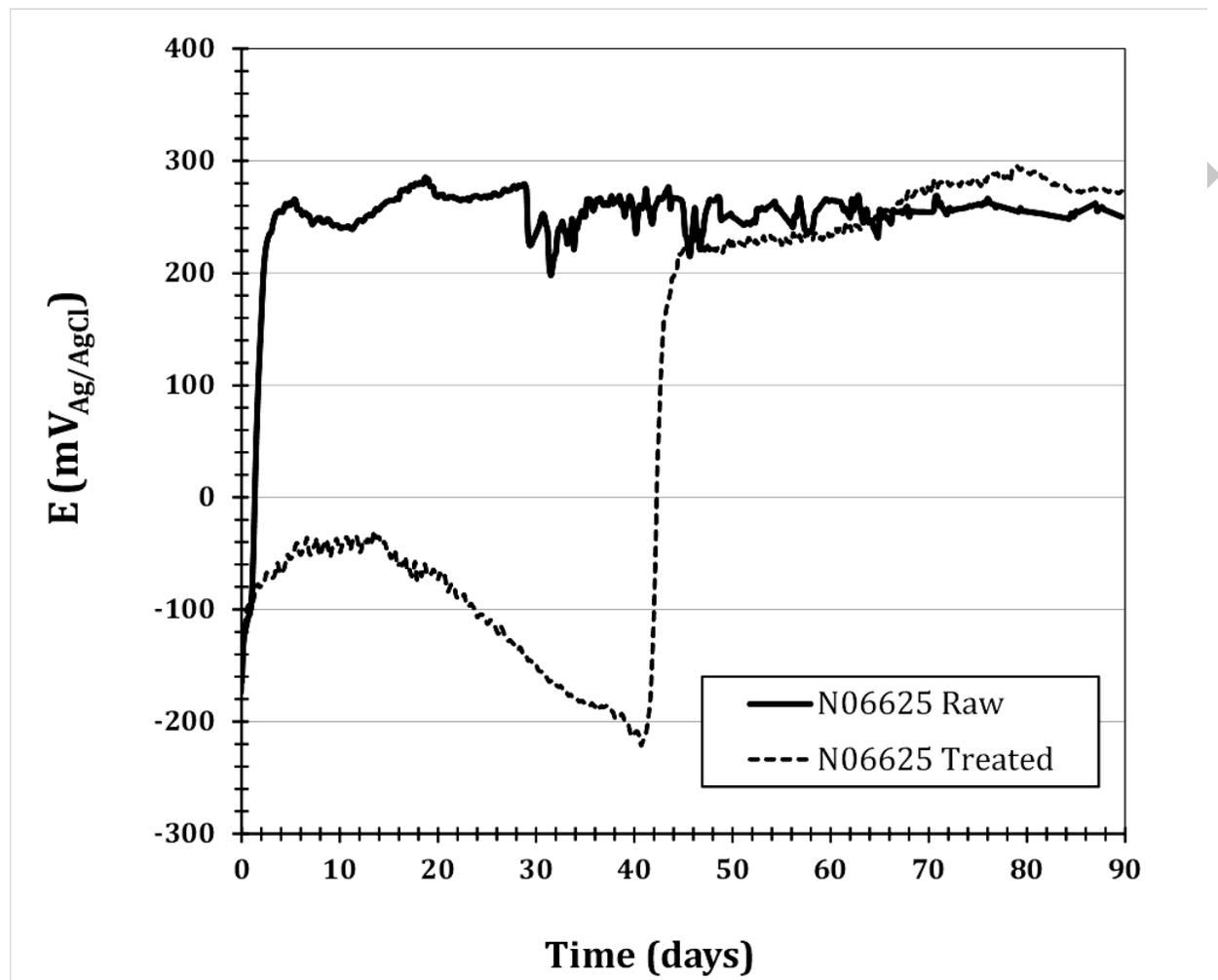
^ddI denotes a deoxyinosine modification.

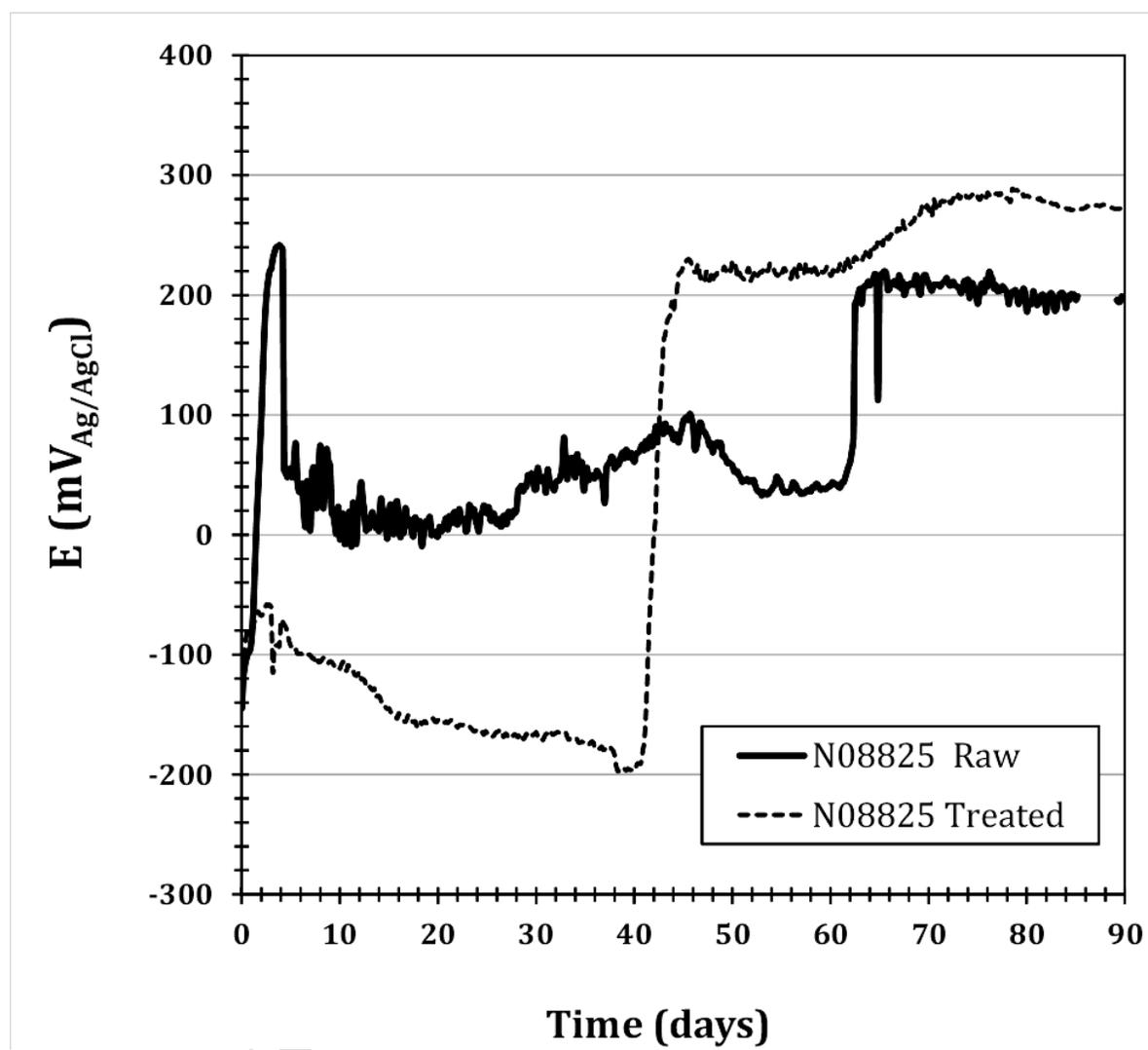


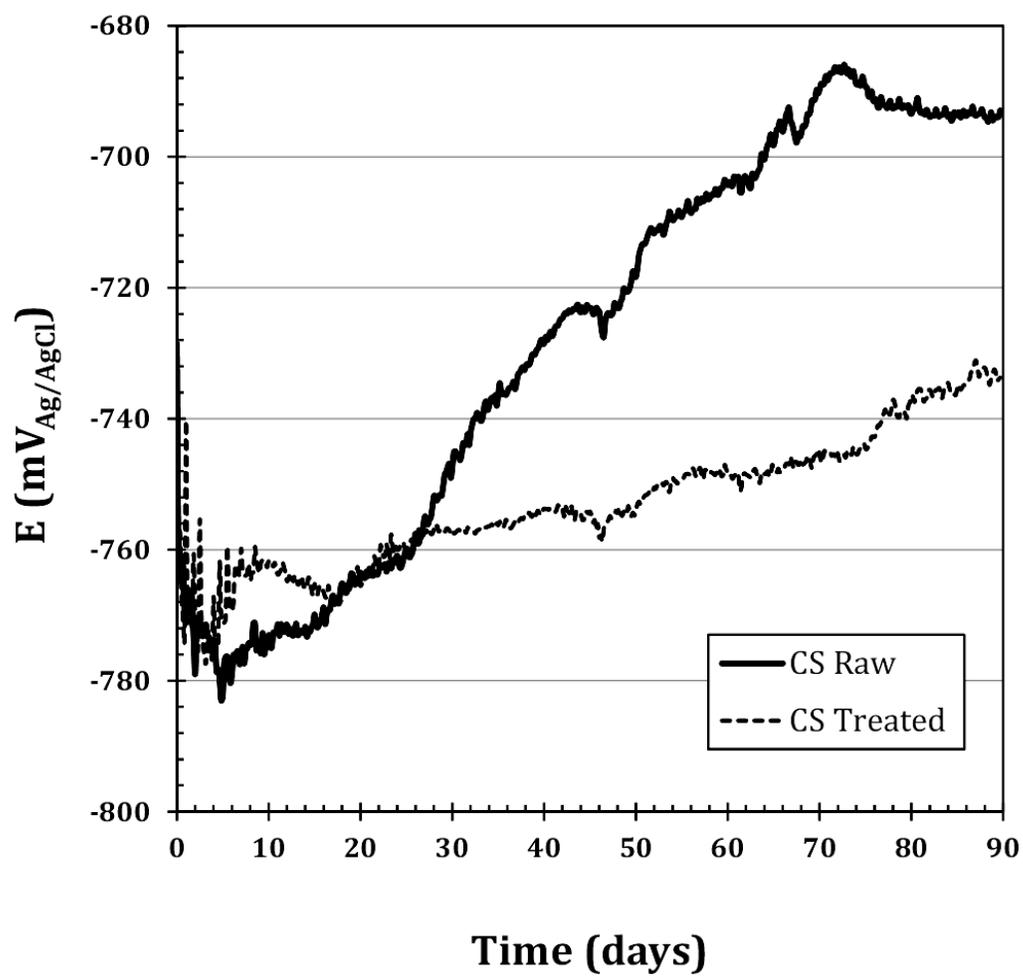


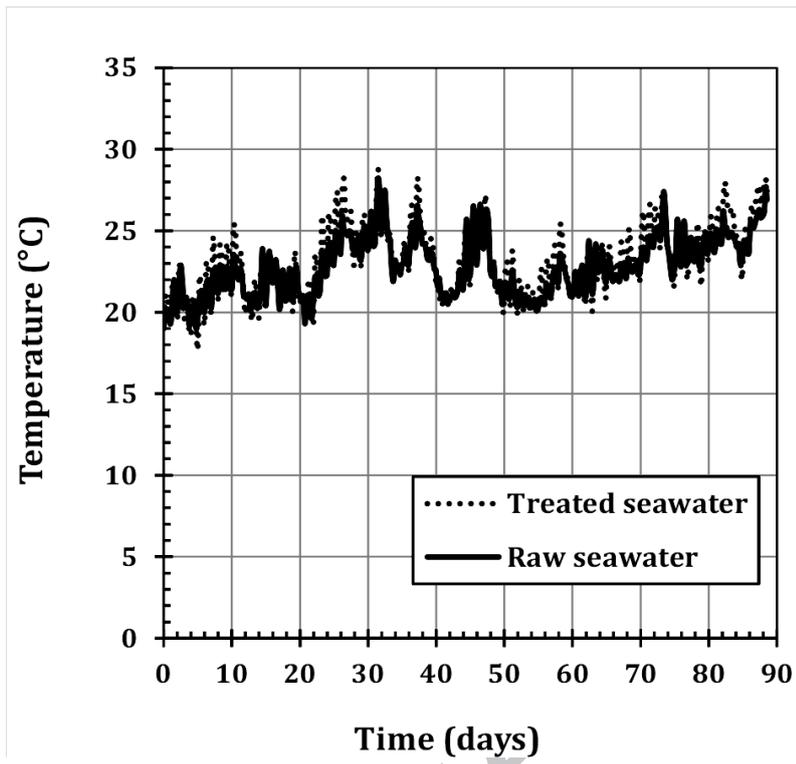


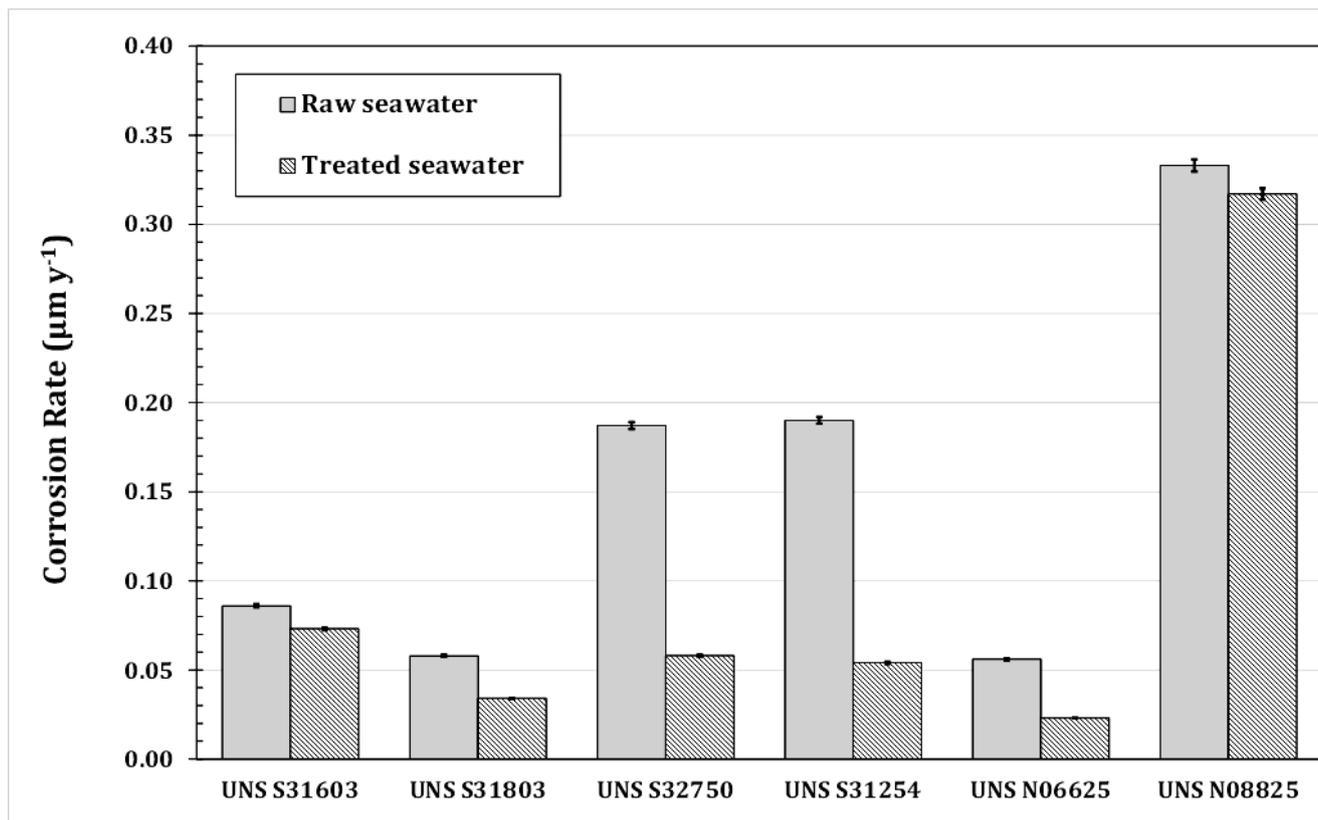


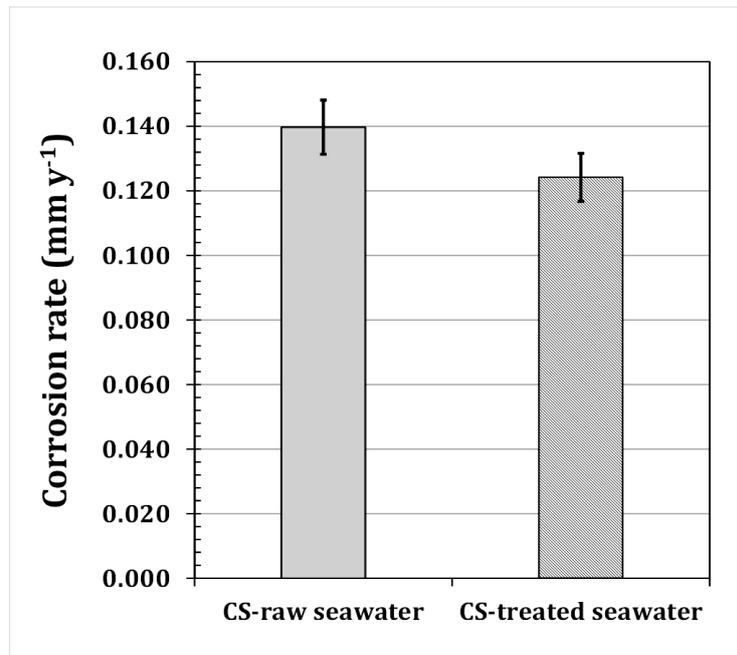


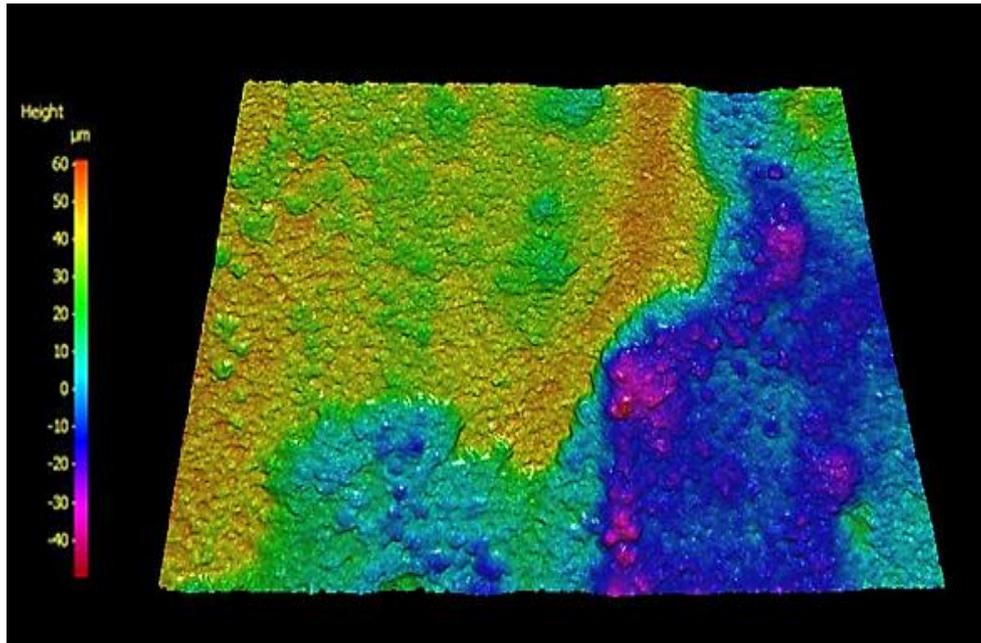


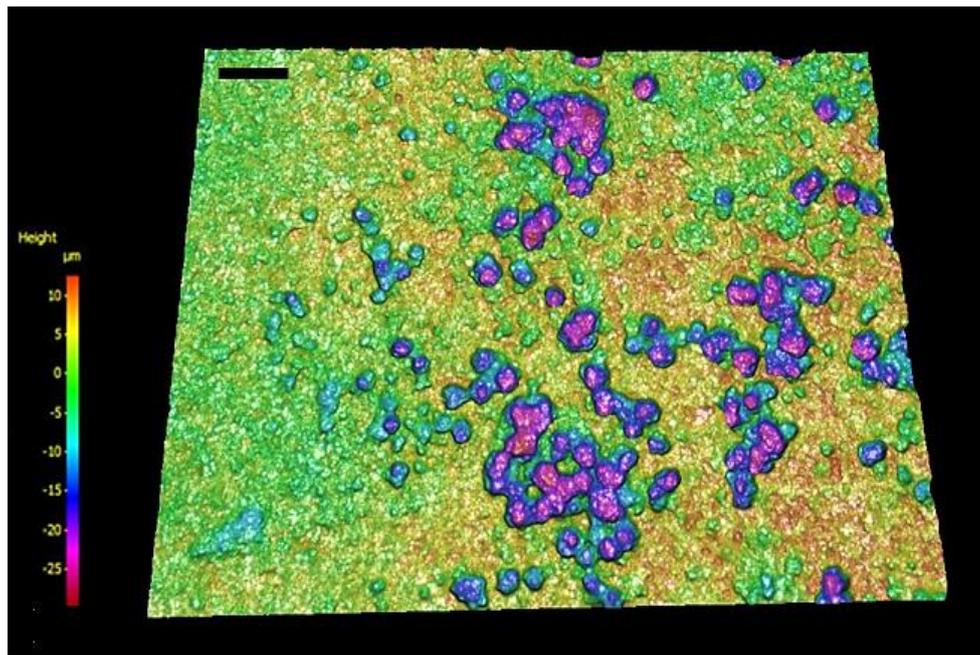


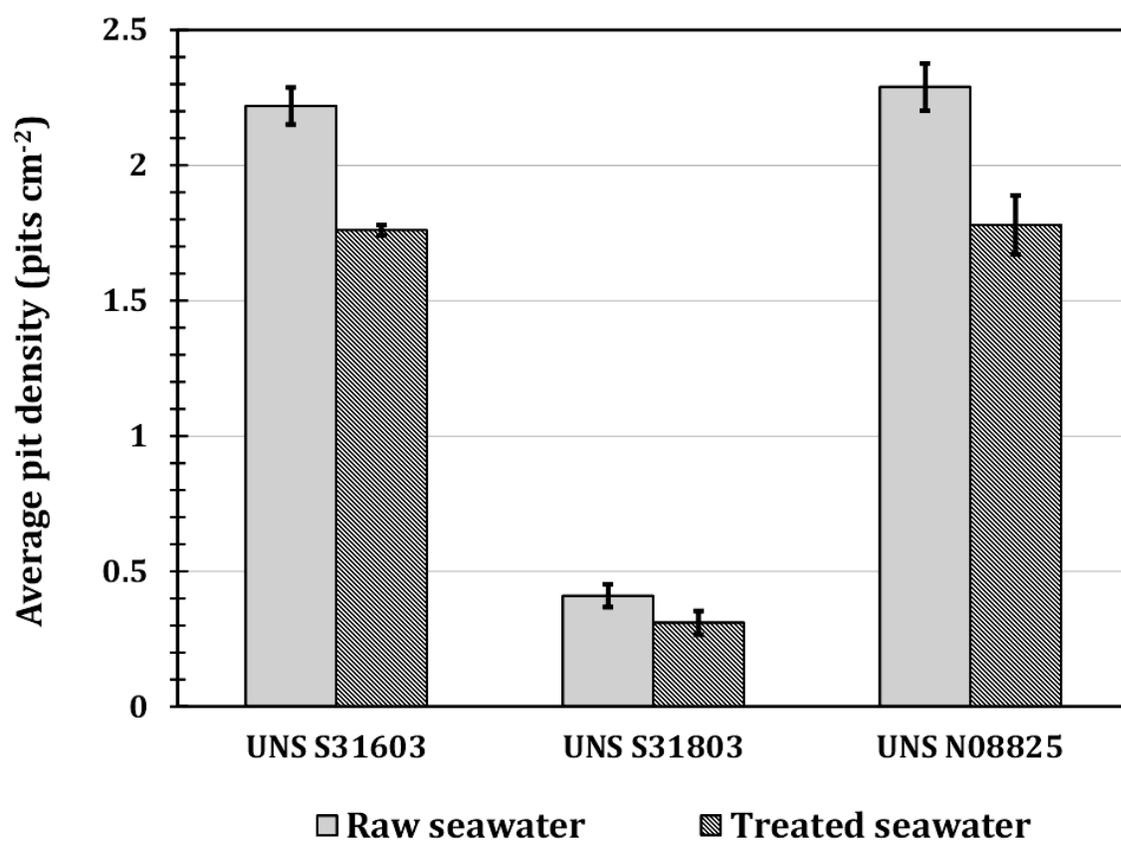




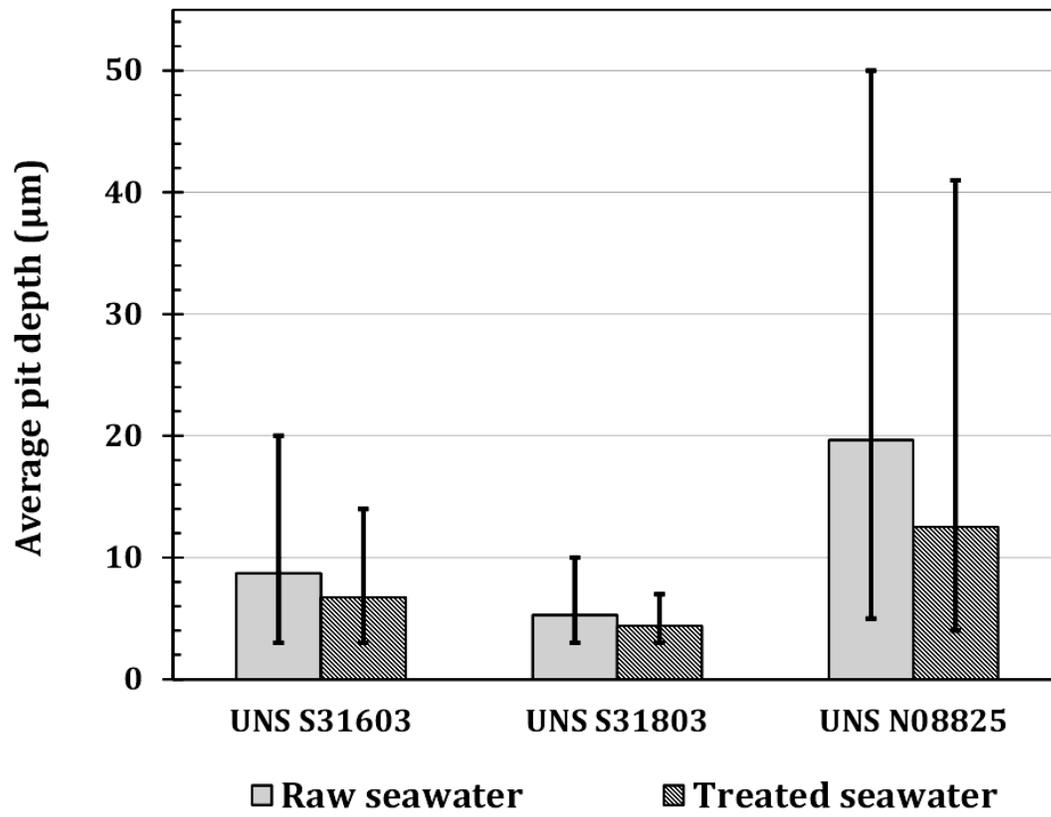


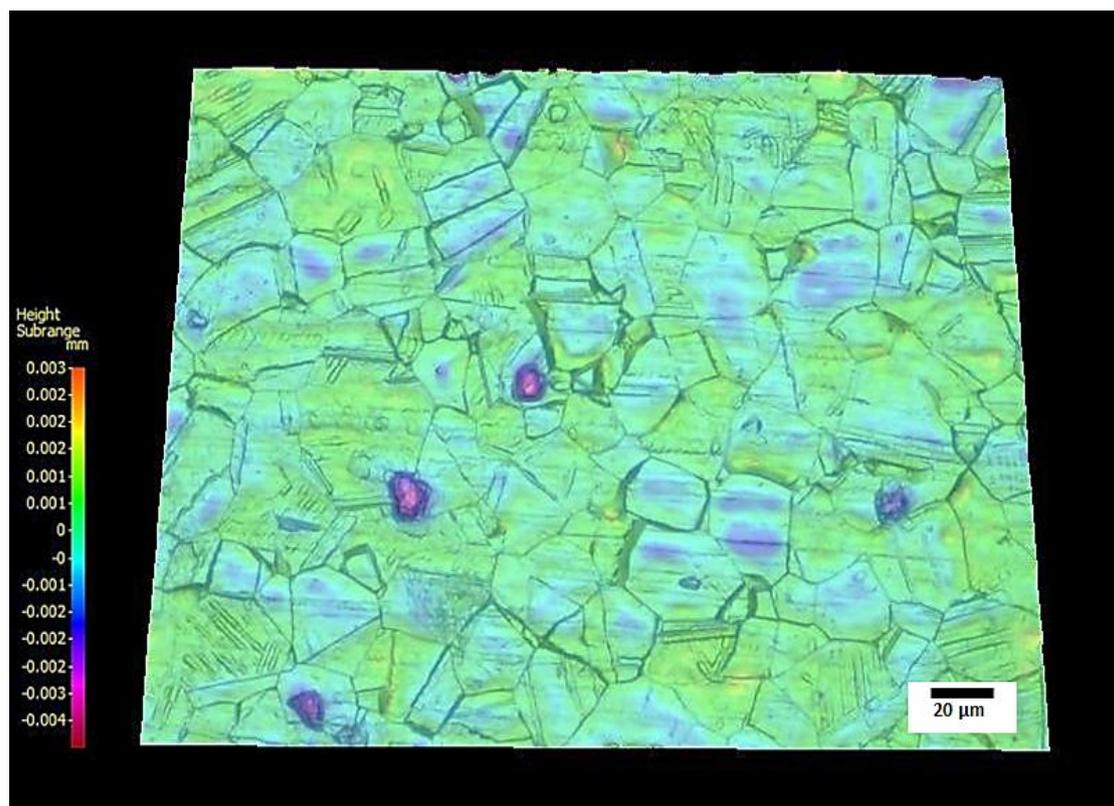


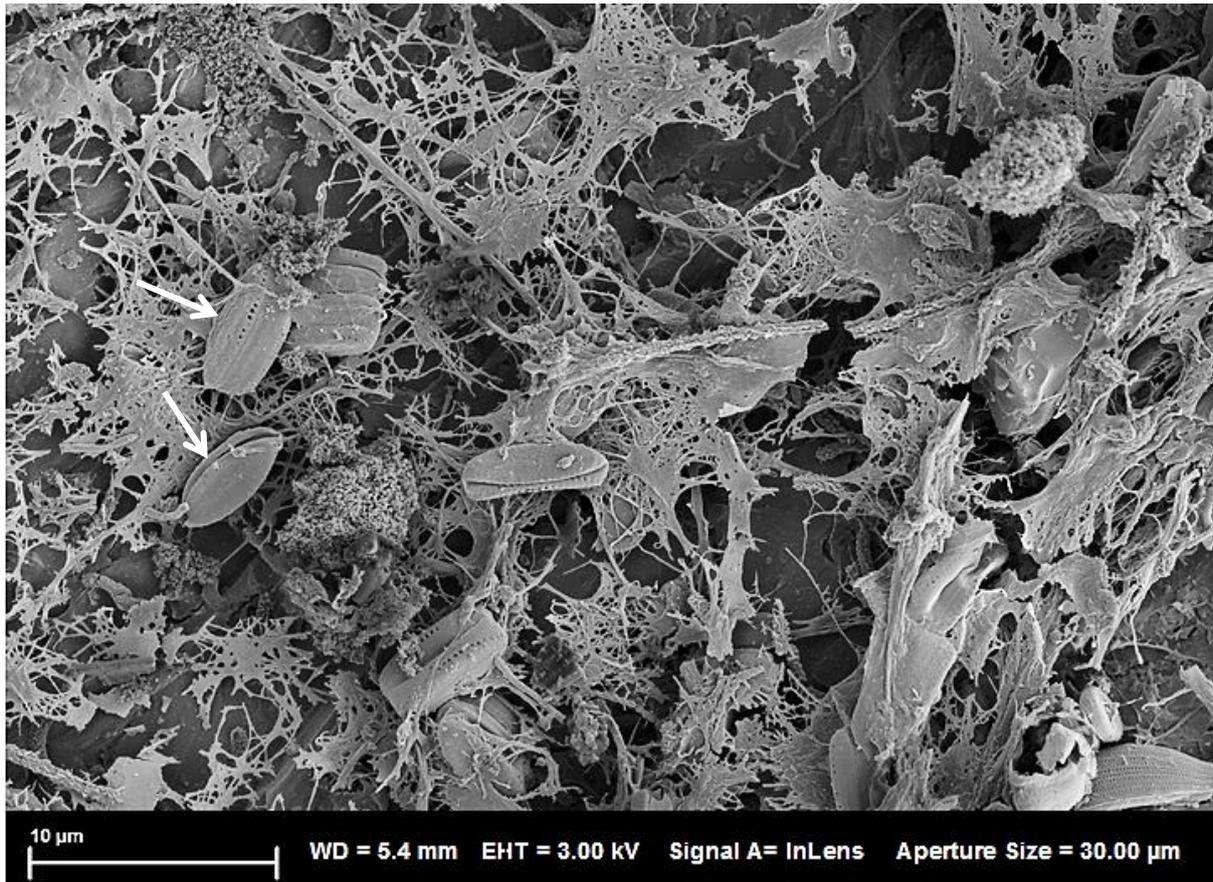




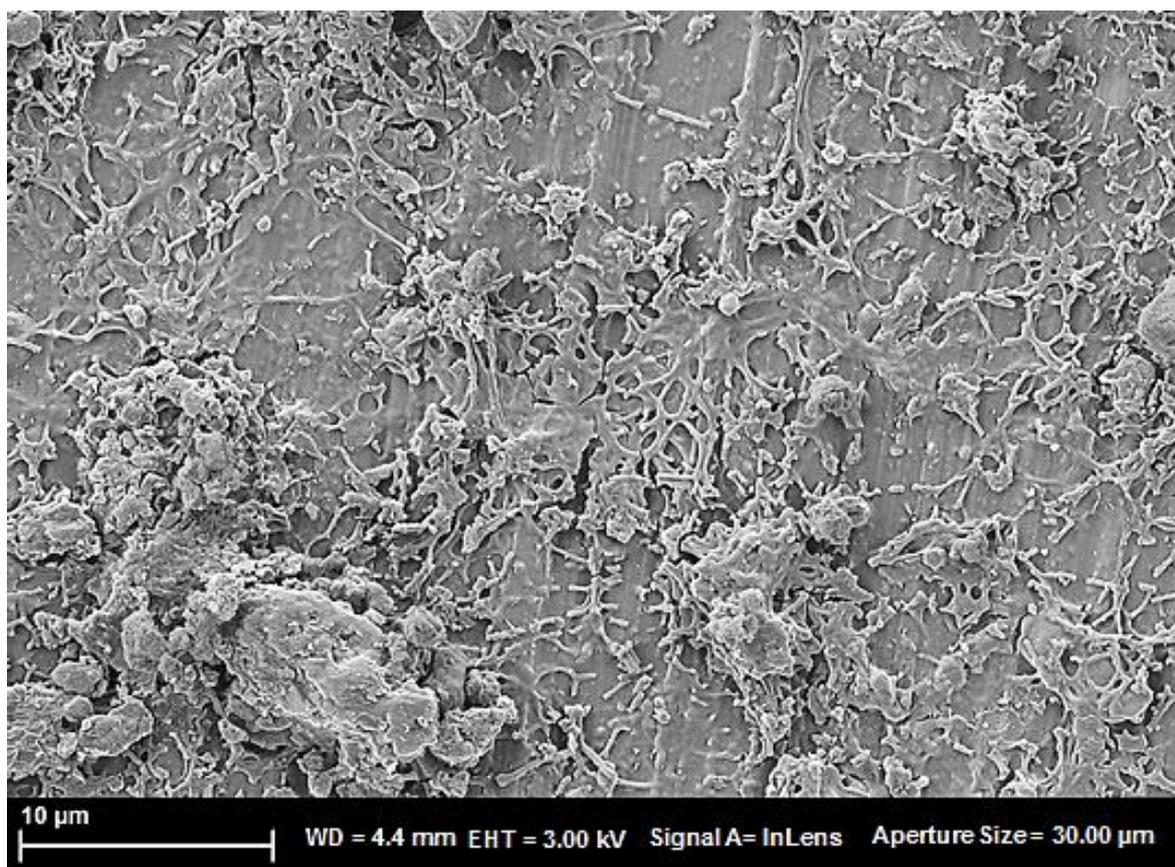
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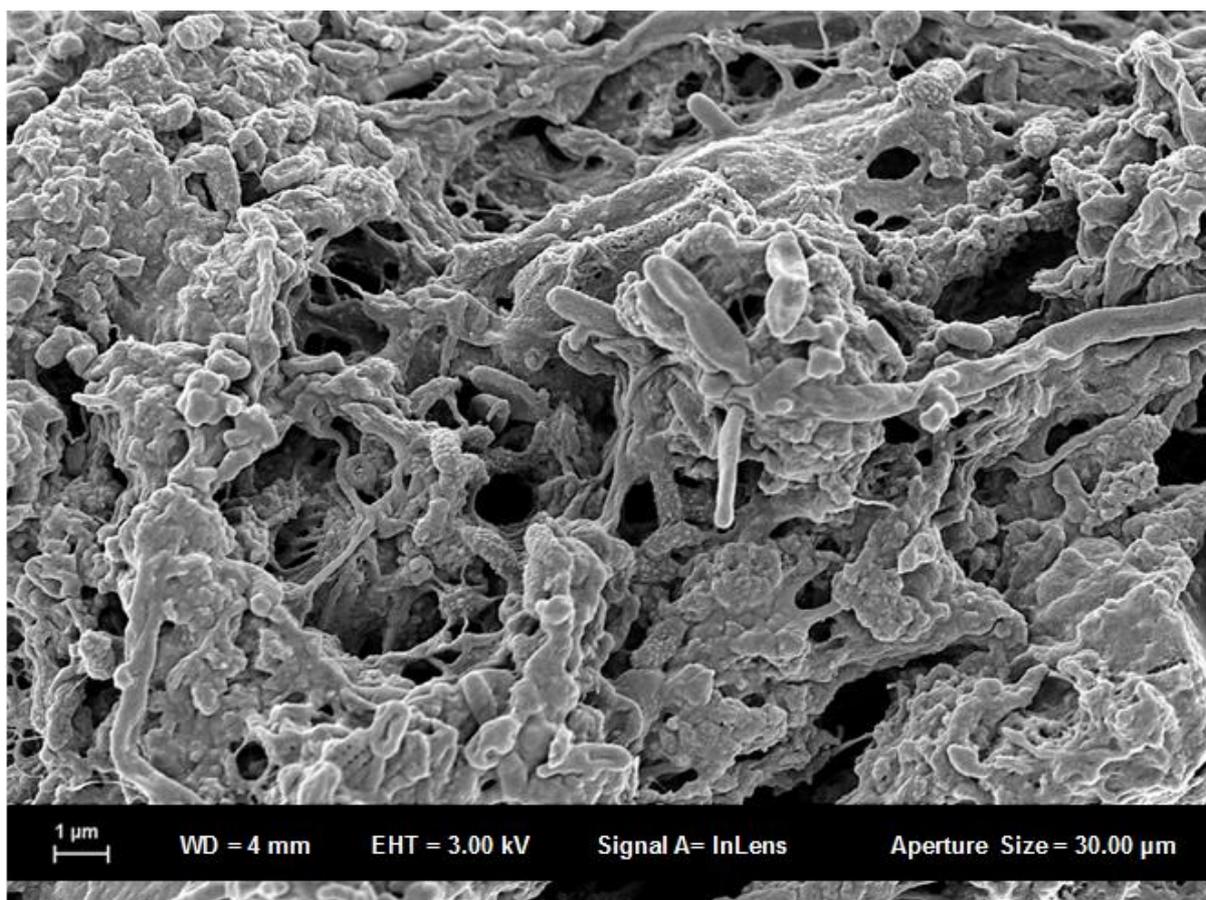




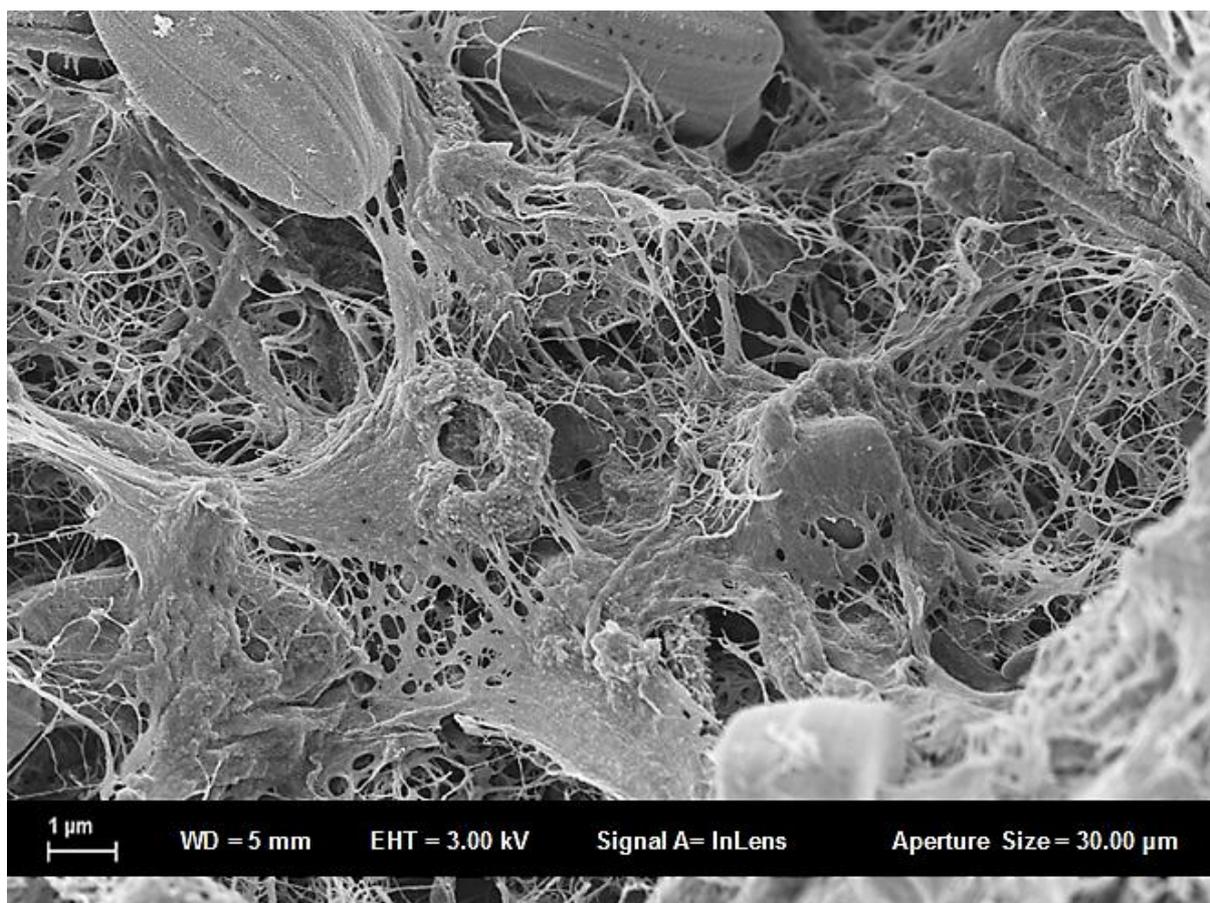
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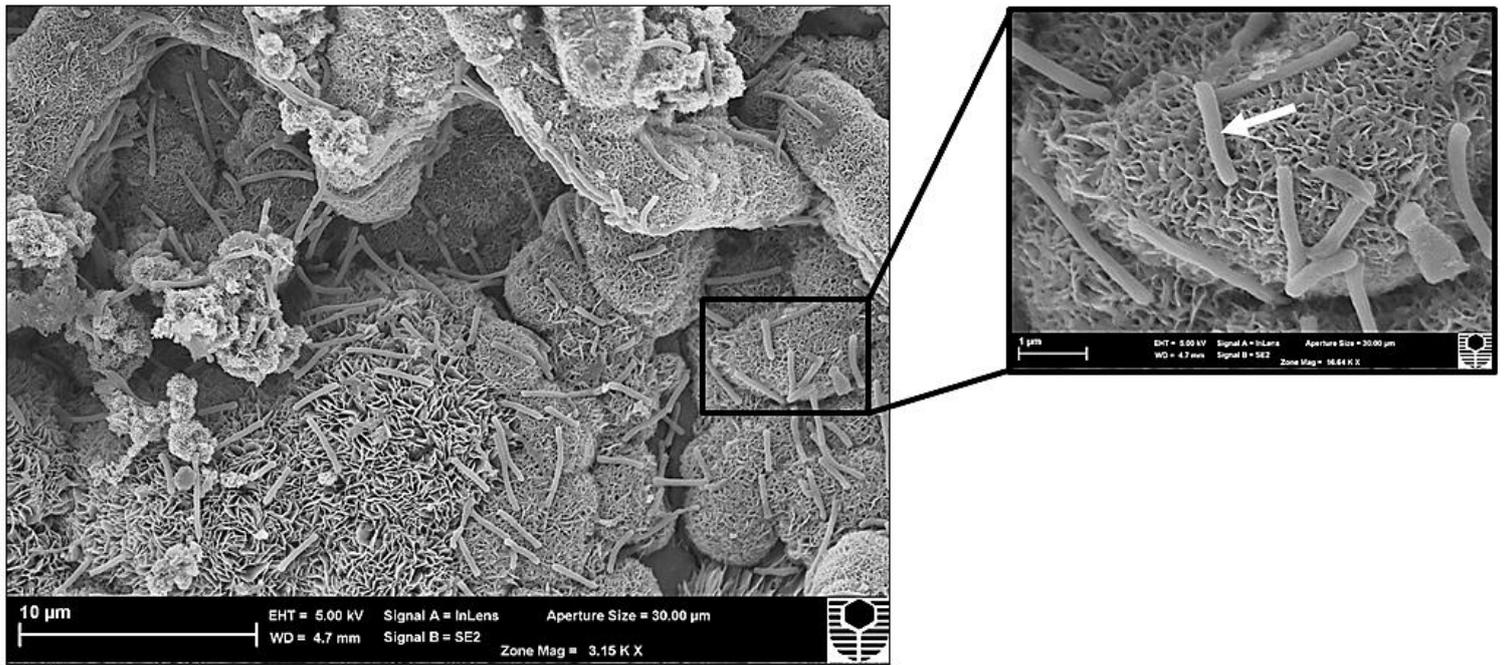
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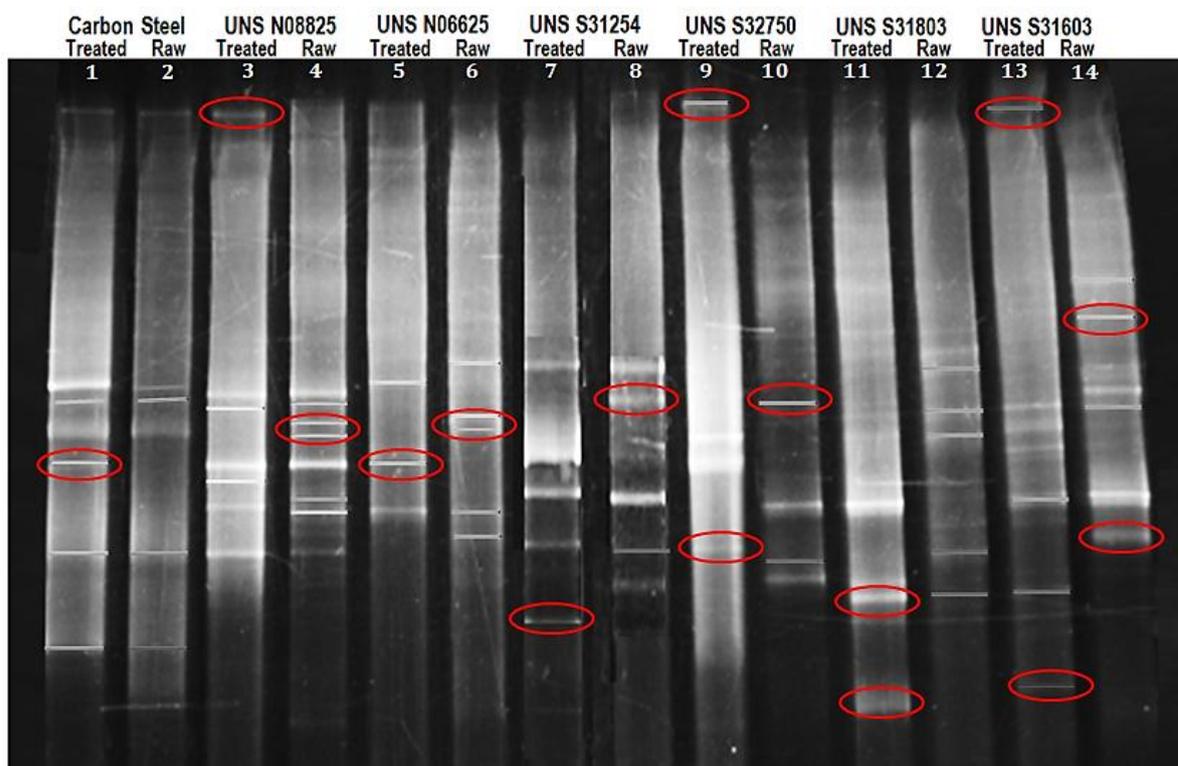
ACCEPTED



ACCEPTED



ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT