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Laboratory Investigation of MIC Due to Hydrotest using Seawater and Subsequent Exposure to Pipeline Fluids with and without SRB Spiking

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ABSTRACT

Microbiologically Influenced Corrosion (MIC) is a major threat to pipelines and storage tanks. Even though MIC during hydrotest itself may be limited due to lack of nutrients and a relatively short duration, biofilms left behind may flourish after the pipeline is commissioned resulting in MIC pitting over the long run. This work investigated MIC in simulated hydrotest with X65 coupons using untreated natural seawater and enriched artificial seawater spiked or not spiked with a laboratory strain of Sulfate Reducing Bacteria (SRB) for up to 90 days. The MIC threat after hydrotested pipes were subsequently exposed to pipeline fluids was also investigated by using simulated pipeline fluids containing a mixture of oil, natural seawater and CO_2 or a mixture of oil, simulated formation water (65000 ppm (w/w) NaCl, 22 mM SO_4^{2-}) and CO_2. Tests were performed at 22ºC and 37ºC in anaerobic vials. The effectiveness of a 30 minute slug of THPS treatment of the coupons right after hydrotest was also evaluated. MIC pitting was observed in simulated hydrotest using enriched artificial seawater spiked with SRB. MIC pitting was also observed on coupons exposed to simulated pipeline fluids after hydrotesting with SRB spiking using either natural seawater or enriched artificial seawater.

Key words: Pipeline, hydrotest, MIC, SRB, CO_2

INTRODUCTION

MIC is a serious threat to pipeline integrity. The practices of water-flooding during enhanced oil recovery, seawater injection break through, and natural increases in produced water all lead to water-wetting condition for pipelines. This promotes microbial growth. Strickland et al.[1] investigated MIC and its mitigation in the Lost Hills, CA oil and water gathering system that suffered a failure 18 months after startup. Samant et al.[2] reported a MIC failure of an offshore 16-inch (40.6 cm) ID pipeline transporting a well fluid containing an oil/gas/water mixture in only 2.5 years. Very recently, Bhat et al.[3] reported that a new 8-inch (20.3 cm) ID pipe carrying oil and produced water failed due to MIC in only 8 months. All these accelerated cases of MIC failure had a continuous water wetting condition. As these water-wet conditions become more common, accelerated pipeline failures will likely be more commonplace.

Before a new pipeline or storage tank is commissioned, they must be assessed for integrity. Hydrotesting is often used to ensure the mechanical strength of the pipeline and check for possible leaks. The testing is always done with a liquid because using a gas such as compressed air presents a safety hazard in case of a leak or rupture. Most of the time, water is chosen due to its low cost and availability. During hydrotest, the fluid fills the pipeline and typically is pressured up to 125% of the design Maximum Allowable Operating Pressure (MAOP) for 8 hours to 10 hours[4]. When the hydrotest is completed, the test fluids will remain in the pipeline as a parking liquid for an extended time until the pipeline is commissioned for service. This period can last for many months.

During this extended time, if the local environmental conditions are favorable to microbes, MIC can occur. Seawater, aquifer water, surface water and produced water are most commonly used in hydrotests and all these water resources may bring contamination of microorganisms. Microbial contamination can also happen in other ways because the system is not sterile[5, 6]. MIC during hydrotest itself may not be a serious problem because of the short period of time and limited nutrients in the hydrotest fluid. However, the biofilms left behind by the hydrotest process can pose a threat. The biofilms may be established before the pipeline is even commissioned. Given the fact that a pipeline may be in use for many decades, a MIC threat initiated from the hydrotest may be a serious concern. The “seed” for a pipeline failure may be planted even before a pipeline is operational. This means a proper hydrotest practice regarding MIC prevention is highly desired.

Filtered or biocide treated seawater is sometimes used for hydrotests intended for offshore installation. Untreated seawater has also been used in some cases. Natural seawater is rich in microorganisms and even biocide treated water can also be a source of microbial contamination[7]. Sulfate reducing bacteria (SRB) are the most common microbes that cause MIC in the oil and gas industry[8]. SRB use sulfate as the terminal electron acceptor and various carbon sources such as volatile fatty acids or even hydrogen gas as electron donors[9]. Seawater typically contains 22 to 30 mM sulfate[10] that is a terminal electron acceptor for SRB metabolism. To prevent oxygen corrosion, oxygen scavengers are added to the hydrotest, which provides an anaerobic environment desirable for SRB. The combined redox reaction is thermodynamically favorable, thus providing energy for SRB growth.

In a fight for survival under poor nutritional conditions, cells in a biofilm can scavenge organic carbons from dead cells[11]. SRB cells can also find maintenance energy by turning to Fe as electron donor, because iron has a similar reduction potential as lactate (-447 mV vs. –430 mV)[9] that is a favored organic carbon for SRB. The two reactions below from Xu et al.[12] show that the combined redox reaction has a positive potential of +247mV. This means the redox reaction is thermodynamically favorable (i.e., energy producing).

$$\text{Anodic:} \quad 4Fe \rightarrow 4Fe^{2+} + 8e^{-} \quad \text{(Iron dissolution)} \qquad E^{o\prime} = -447 \text{ mV} \qquad (1)$$

$$\text{Cathodic:} \quad SO_4^{2-} + 8H^{+} + 8e^{-} \rightarrow HS^{-} + OH^{-} + 3H_2O \quad \text{(BCSR)} \qquad E^{o\prime} = -200 \text{ mV} \qquad (2)$$

Xu et al.[12] performed an SRB starvation experiment to test the SRB corrosion mechanism based on SRB bioenergetics. Their data showed that an established SRB biofilm starved of organic carbon was

more aggressive against carbon steel. This contradicted conventional wisdom that a well-fed biofilm is more aggressive. It is worth noting that the starvation tests started with the same SRB biofilms. Starting with different biofilm densities on coupon surfaces are not good for such starvation tests because SRB pitting depends on the number of SRB cells right on the iron surface[13].

Lab tests to reproduce actual hydrotest field conditions are difficult because of the high pressure and large liquid volume requirements. Cheung et al.[14] showed that SRB grew at both 1 atm and 200 atm even under biocide attack. Rosnes et al.[15] reported that SRB isolated from a North Sea oil field grew and tolerated up to 300 bar of pressure. Thus, it is not detrimental to use 1 atm to simulate MIC attack although the effect of pressure on MIC pitting remains a worthwhile topic for future investigations. Biocides are often used to prevent MIC during hydrotest[4]. However, because of strict environmental regulations, biocide treatment of hydrotest fluid is expensive. Without biocide treatment, and sometimes even with biocide treatment, MIC remains a potential threat. It is useful to perform a scientific study on MIC threat in hydrotest.

This work investigated the MIC threat during hydrotest using untreated seawater, the effectiveness of a simulated slug biocide treatment, and the MIC threat after hydrotested pipes were subsequently exposed to pipeline fluids.

EXPERIMENTAL PROCEDURE

Experimental conditions during hydrotest

Seawater collected from Gulf of Mexico was used as the hydrotest fluid. The filtered natural seawater was quite clean with limited organic carbon content and microbes. The TOC (Total Organic Carbon) content was found to be 0.4 ppm using a wet oxidation total organic carbon analyzer. Because limited liquid volume and coupon surface area were used for testing, there was a chance that the opportunistic biofilm establishment might not occur during lab tests. Therefore, *Desulfovibrio alaskensis* (ATCC 14563, a marine SRB) was used to spike the seawater (to achieve an initial SRB concentration of 10^6 cells/ml right after inoculation) to simulate worst-case microbial contamination during hydrotest.

These tests were carried out in 120 ml anaerobic vials where each vial contained 100 ml liquid. All liquids were deoxygenated including natural seawater with N_2 sparging prior to use. A glove box was deoxygenated using filtered N_2 gas to provide an anaerobic environment for manipulations. Anaerobic vials have a propensity to allow small amounts of oxygen to enter during long-term tests despite the septum seal with aluminum cap. To eliminate oxygen ingress, 100 ppm of cysteine was added into each vial as an oxygen scavenger.

Rectangular shaped X65 steel coupons with a surface area ranging between 4.5-4.9cm^2 were placed in the anaerobic vials at a roughly 30^o angle. Only the top of the coupon surface was exposed to the headspace. All other surfaces were painted with inert Teflon. The ratio of exposed coupon area to liquid volume matched that in a 32” to 35” ID pipe. The coupons were polished progressively with 200, 400 and 600 grit abrasive papers.

Cell counts

Planktonic SRB cell count was measured using a hemocytometer under an optical microscope at 400X. Only viable, motile cells were counted. To determine the sessile cell count, the coupon surface was first rinsed with distilled water (deoxygenated) to remove the planktonic cells. Then the coupon and 10 ml of water were combined in a sterile test tube. The coupon surface was scraped using a surgical knife until all visible black FeS film was removed. After sonicating for 30 seconds and vortexing for another 30 seconds, the mixture of sessile cells in water was tested using an SRB test kit. The kit contained a test vial filled with a solid SRB medium. A brush-like dipstick was used to gather cells in the cell suspension and then inserted into the solid medium. The time required for the black color to appear around the dipstick vs. standard times provided by the vendor was indicative of the SRB concentration.

Measurement of sulfate concentration

Sulfate concentrations were measured using a simple turbidimetric method described by Kolmert et al.[16] The method uses barium ion to precipitate sulfate.

Weight loss and SEM observation of biofilms and pits

Biofilms and pits on the coupon surface were observed using SEM. The coupon preparation procedure to observe the biofilms was described by Wen et al.[17]. The sessile cells on the coupons were not evenly distributed. An SEM image was taken in an area with a dense cluster of sessile cells. Following SEM imaging, the biofilm and the applied gold film was subsequently removed from the coupon surface with Clark's solution (per ASTM G1-03 [18]) and then weighed. An SEM image was taken in the area with the largest pits.

Hydrotest using natural seawater and enriched artificial seawater with and without SRB spiking

Two duplicate coupons were added to each anaerobic vial. The native SRB concentration in the natural seawater was examined using the SRB test kit (Biosan Sani-Check[1] Product #100). To simulate a contamination condition, some vials were spiked with 10^6 cells/ml SRB. The tests were run at 37°C and 22°C, respectively. The longest test duration was 90 days. Because the natural seawater lacked organic carbon for microbial growth, enriched artificial seawater was also used to simulate worst-case scenario, such as produced water with much more organic carbon. Additionally, artificial seawater is well defined and more easily reproduced. The enriched artificial seawater composed of a salt mix (intended for marine aquarium) supplemented with 125 mg $Fe(NH_4)_2(SO_4)_2$, 4.5 ml sodium lactate (60 wt% syrup) and 1 g yeast extract to make 1 L of artificial seawater. The detailed composition is listed in Table 1.

Table 1

Comparison between natural seawater and artificial seawater used in this work.* 36 g commercially available salt mix (intended for marine aquarium) supplemented with 125 mg $Fe(NH_4)_2(SO_4)_2$, 4.5 ml sodium lactate (60 wt% syrup) and 1 g yeast extract to make 1 L of artificial seawater)

		Component concentration								
	Salinity (in 1000 ppm)	Na^+	Mg^{2+}	Ca^{2+}	K^+	Sr^{2+}	Cl^-	SO_4^{2-}	BO_3^{3-}	CO_3^{2-} HCO_3^-
Natural Seawater	35	470	53	10.3	10.2	0.09	550	28	0.42	1.90
Enriched Artificial Seawater	29.7	462	52	9	9.4	0.19	521	23	0.43	1.90

*All in millimoles per kg, except salinity

Exposure to simulated pipeline fluid (oil, natural seawater and CO_2) using coupons from simulated hydrotest in natural seawater with and without biocide treatment

After removing the coupons from the hydrotest which had natural seawater spiked and not spiked with SRB for 30 days, the coupons were washed with distilled water (deoxygenated) to remove planktonic cells from the coupon surfaces. Some coupons were then immersed in a 300 ppm (active) THPS (Tetrakis Hydroxymethyl Phosphonium Sulfate) biocide solution for 30 min to simulate a slug biocide treatment, while some coupons were not treated. The washing and biocide treatment were carried out in an anaerobic glovebox filled with 1 bar CO_2. To simulate post commissioning pipeline conditions, coupons were then put into vials containing 100 ml deoxygenated mixture of oil (LVT-200 oil[2]) and natural seawater (sparged with CO_2) for one hour. The ratio of oil/water was 1:10 (v/v) throughout this work when such a mixture was involved. The vials were finally sealed using rubber septa and aluminum

[1] Trade name

[2] Trade name

caps in the glovebox. Additional measures were taken to ensure anaerobic conditions using was to cover the cap and bottleneck area of each vial.

Exposure to simulated pipeline fluid (oil, simulated formation water and CO_2) using coupons from simulated hydrotest in enriched artificial seawater without biocide treatment

The procedure was similar to the previous section using natural seawater. Enriched artificial seawater spiked with 10^6 cells/ml SRB was left in anaerobic vials containing X65 coupons for 15 days to simulate hydrotest. After the 15 days, the coupons were taken out and exposed to a mixture of oil and simulated formation water (containing 65000 ppm NaCl, 22 mM SO_4^{2-}) and CO_2 for another 15 days. The coupons were then harvested and examined for biofilms and pits.

RESULTS AND DISCUSSION

Hydrotest using natural seawater without spiking

The sulfate concentration of the Gulf Mexico seawater sample was found to be 24 mM. It is within the sulfate concentration range in typical natural seawater[10]. Table 2 shows that without spiking, native sulfate reducer (both planktonic and sessile) cells were found at low concentrations after 90 days of incubation at 22°C, but no SRB were found for the 37°C incubation temperature. This finding can be confirmed by the SEM images in Figure 1. A biofilm containing sessile cells was observed for the 22°C coupon (Figure 1B) while none were seen for 37°C (Figure 1A). No pitting corrosion was found at both 37°C (Figure 2A) and 22°C (Figure 2B) after 90 days without SRB spiking. The SEM images in Figure 2 (for bare metal surfaces after biofilm removal) correspond to those in Figure 1 (for biofilms). The results seem to suggest that hydrotest using clean filtered natural seawater in a limited time frame by itself may be relatively safe from MIC because the seawater is low in TOC and native microbial cell count. However, this may not be foolproof because any microbes left behind by hydrotest can still flourish when nutrients are introduced by the pipeline fluid after the pipeline is commissioned.

Table 2

Planktonic cell count in the bulk liquid and sessile cell counts on coupon surfaces measured using the SRB test kit for coupons exposed to the natural seawater during hydrotest for 30 days and 90 days, respectively. (The initial cell concentration of unspiked natural seawater was less than 10 cells/ml.)

Conditions	Planktonic Cell Count (30 days) (cells/ml)	Planktonic Cell Count (90 days) (cells/ml)
Spiked with 10^6 SRB cells per ml at 37°C	≥10^4	＜10
Without spiking at 37°C	＜10	＜10
Spiked with 10^6 SRB cells per ml at 22°C	≥10^4	≥10^2
Without spiking a 22°C	≥10^2	＜10
	Sessile Cell Count (30 days) (x 2.1 cells/cm^2)**	Sessile Cell Count (90 days) (x 2.1 cells/cm^2)**
Spiked with 10^6 SRB cells per ml at 37°C	≥10^3	＜10
Without spiking at 37°C	＜10	＜10
Spiked with 10^6 SRB cells per ml at 22°C	≥10^4	≥10^2
Without spiking at 22°C	≥10^2	＜10

**The conversion factor 2.1 reflects the fact that for a biofilm on a 4.7 cm^2 coupon surface, 10 ml water was used to re-suspend the removed sessile cells for testing using the SRB test kit.

Hydrotest using natural seawater spiked with SRB

Figure 3A shows that when spiked with 10^6 cells/ml SRB, the planktonic cell counts reached a small peak and then declined to the inoculum level on day 7 for both 22°C and 37°C. After day 7, the cell counts kept decreasing and went below the inoculum level due to a lack of organic carbon in the natural seawater. Due to the limited carbon source in the natural seawater, cells grew for less than 5 days. Cell counting under microscope at 400X magnification had a detection limit of $5x10^4$ cells/ml minimum. Motile cells were easily seen at 400X. Due to the low cell counts after day 15, the SRB test kit had to be used to enumerate the cells. In Table 2, after 30 days, the planktonic cell counts indicated by the SRB test kit show that SRB cells present at a concentration of 10^4 cells/ml were still alive at both 37°C and 22°C after 30 days. However, only 10^2 cells/ml SRB were still detected after 90 days at 22°C. At 37°C, the cell count was below the SRB test kit's detection limit after 90 days. Figure 3B will be discussed in the next section with artificial seawater results.

It is well-known that sessile cells are directly responsible for MIC pitting. In Figures 1C and 1D, sessile cells can be clearly observed after 90 days even at a low organic carbon level in the natural seawater spiked with SRB. Table 2 and Figures 1C and 1D all indicate that there were more sessile cells at 22°C than at 37°C. Figures 2A and 2B (both without SRB spiking) show that the coupon surfaces were rough, but do not appear to be typical of MIC pitting. For coupons with SRB spiking, MIC pitting corrosion was clearly observed at both 37°C (Figure 2C) and 22°C (Figure 2D). The largest pits found at 37°C were larger than those seen at 22°C. Figure 4A shows the measured weight loss for samples spiked with 10^6 cells/ml SRB at 22°C was larger than those observed at 37°C. Figure 4A also indicates that at 22°C and 37°C, the weight loss for spiked coupons were considerably larger than the unspiked coupons. Figure 4B, a chart of remaining sulfate concentrations as a function of time, shows that sulfate consumption increased over time and more sulfate was consumed at 22°C than at 37°C in both spiked and unspiked tests. The data in Figure 4 also prove that spiking increased sulfate consumption as expected because there were more SRB cells present.

Hydrotest using enriched artificial seawater spiked with SRB

For samples spiked with 10^6 cells/ml SRB, Figure 3B shows that planktonic cell counts (under microscope) reached a peak and eventually declined to the inoculum level on day 30 for both 22°C and 37°C. The planktonic and sessile cell counts on day 30 were quantified using a microscope and were consistent with SRB test kit results as shown in Table 3. Because of the added nutrients in the enriched artificial seawater, 1 $\log_{10}$ more planktonic cells at both temperatures were present on day 30 compared with natural sweater. There were also 1 $\log_{10}$ more sessile cells at 37°C on day 30 compared with the natural seawater.

In the SEM images in Figures 5A and 5C, numerous sessile cells can be clearly seen on the 30-day coupon surfaces. Pitting corrosion was observed at both 22°C (Figure 5B) and 37°C (Figure 5D). In Figure 5, the largest pits found at 22°C were larger than those seen at 37°C (roughly 10 microns v. 5 microns in surface dimensions).

Table 3

Planktonic cell count and sessile cell count data on 30th day in enriched artificial seawater spiked with 10^6 cells/ml SRB

Temperature	Planktonic Cell Count (cells/ml)	Sessile Cell Count (x 2.1 cells/cm^2)
37°C	≥10^5	≥10^4
22°C	≥10^5	≥10^4

Exposure to simulated pipeline fluid (oil, seawater and CO_2) using coupons from simulated hydrotest in natural seawater with and without biocide treatment

Pipeline fluids may contain organic carbons (including volatile fatty acids that are favored by SRB) from oil, CO_2, and water that may come from the practice of water-flooding. It was reported that some SRB can even use CO_2 autotrophically[9] similar to methanogens. MIC may accelerate CO_2 corrosion by interfering with the formation of the protective iron carbonate film. Thus, MIC involving CO_2 should be an important research topic.

After 30 days in the simulated hydrotest using natural seawater, the numbers of sessile cells on the coupon surface stayed at 10^3 to 10^4 cells/ml in the tests with SRB spiking. The coupons after hydrotest with natural seawater were removed. Some coupons were treated with 300 ppm (active) THPS treatment and some were not. The coupons were subsequently exposed to a mixture of oil and natural seawater containing CO_2 for 15 days.

After the coupons were exposed to simulated pipeline fluid for 15 days, the SRB test kit did not detect planktonic or sessile cells on coupons treated or untreated with THPS prior to the exposure. The primary reason could be that the cells died from a lack of organic carbon in the natural seawater. It was also possible that a lower pH (around pH 5-6 in Figures 6) in the simulated pipeline fluid containing CO_2 was not favorable. The SEM images in Figure 7 confirm that there were no sessile cells left.

Exposure to simulated pipeline fluid (oil, simulated formation water and CO_2) using coupons from 15 days of simulated hydrotest in enriched artificial seawater

Following hydrotest conditions, the coupons were exposed to simulated pipeline fluid without a prior biocide treatment. The coupons were examined under IFM (Infinite Focus Microscope) to find deepest pits after exposure to simulated pipeline fluid for 15 days. In Figures 8A and 8C, sessile cells are clearly visible. Pitting corrosion was observed at both 22°C (Figure 8B) and 37°C (Figure 8D). The largest pits found at 22°C were larger than those at 37°C. Large and shallow pits can be found on coupons for both temperatures. The largest pit depth found at 22°C was about 15 microns (Figure 9C1) while it was 20 microns at 37°C (Figure 9C2).

CONCLUSIONS

1. Even though native sulfate reducers were present in the Gulf of Mexico seawater, no MIC was observed following 90 day simulated hydrotests. This wass likely due to low cell populations and low TOC in the natural seawater.

2. When the cell concentration in the natural seawater was increased to 10^6 cells/ml SRB by spiking, both planktonic and sessile cells were detected after 90 days of simulated hydrotest at 22°C, while only sessile cells were detected at 37°C under SEM (but not with the SRR test kit).

3. When the simulated hydrotest fluids were enriched with artificial seawater, MIC pitting was observed after just 30 days of simulated hydrotest at both 22ºC and 37ºC. The largest pit at 22ºC has roughly twice the (surface) diameter as that at 37ºC.

4. When exposed to a mixture of oil, natural seawater, and CO_2 for 15 days following 30 days of simulated hydrotest in natural seawater with and without THPS treatment, no planktonic or sessile cells were detected. Thus, the natural seawater could not provide the nutrients needed to sustain microbial growth.

5. When exposed to a mixture of oil, simulated formation water, and dissolved CO_2 for 15 days following simulated hydrotest in enriched artificial seawater for 15 days without biocide treatment, pits were observed at both at 37°C and 22°C. The largest pit depth was larger at 37°C (20 microns) than at 22°C (15 microns).

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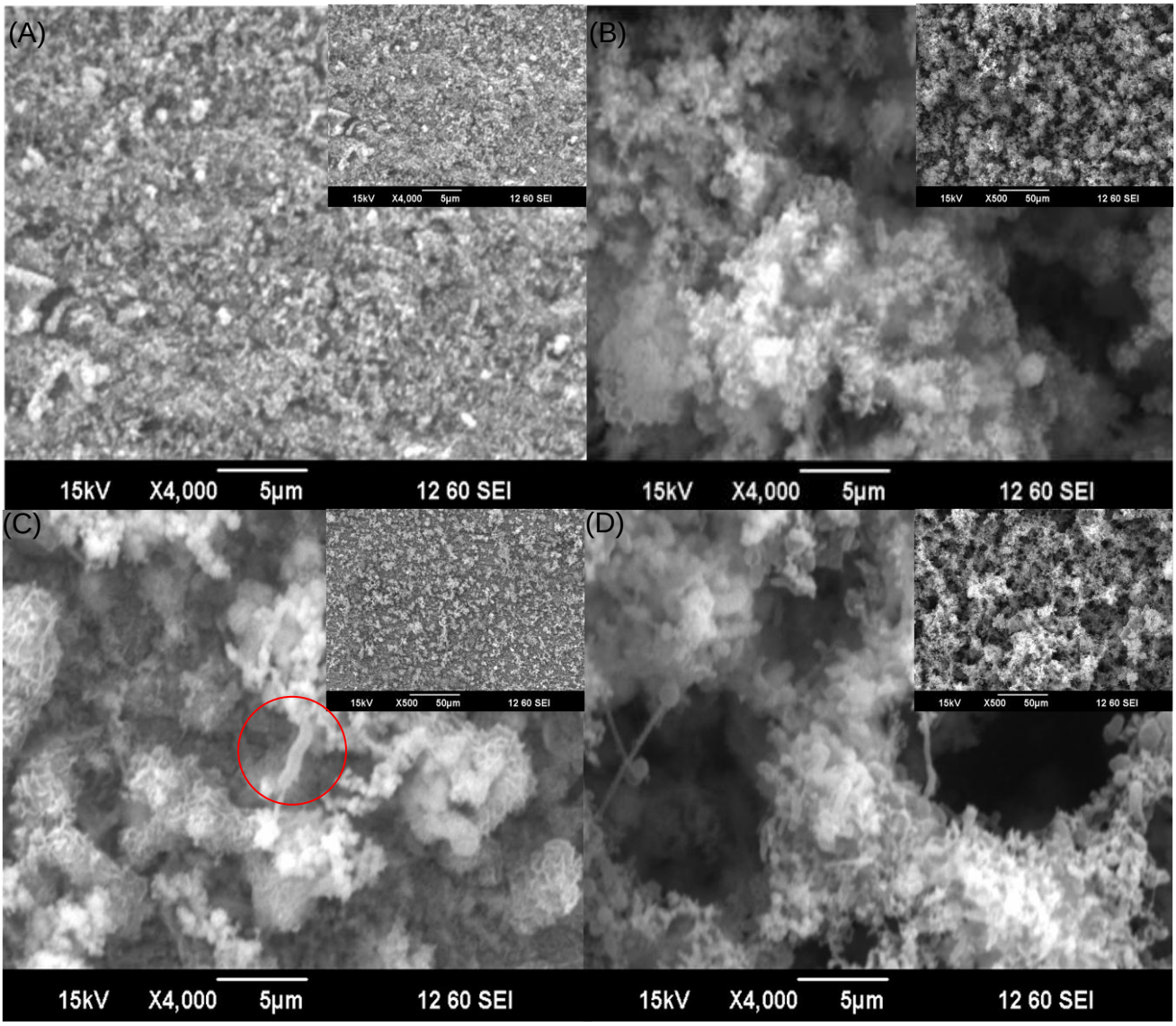

Figure 1. SEM biofilm images on X65 coupon surfaces. Coupons were from 90-day simulated hydrotest in natural seawater: (A) without SRB spiking at 37ºC, (B) without SRB spiking at 22ºC, (C) spiked with 10^6 cells/ml SRB at 37ºC, and (D) spiked with 10^6 cells/ml SRB at 22ºC.

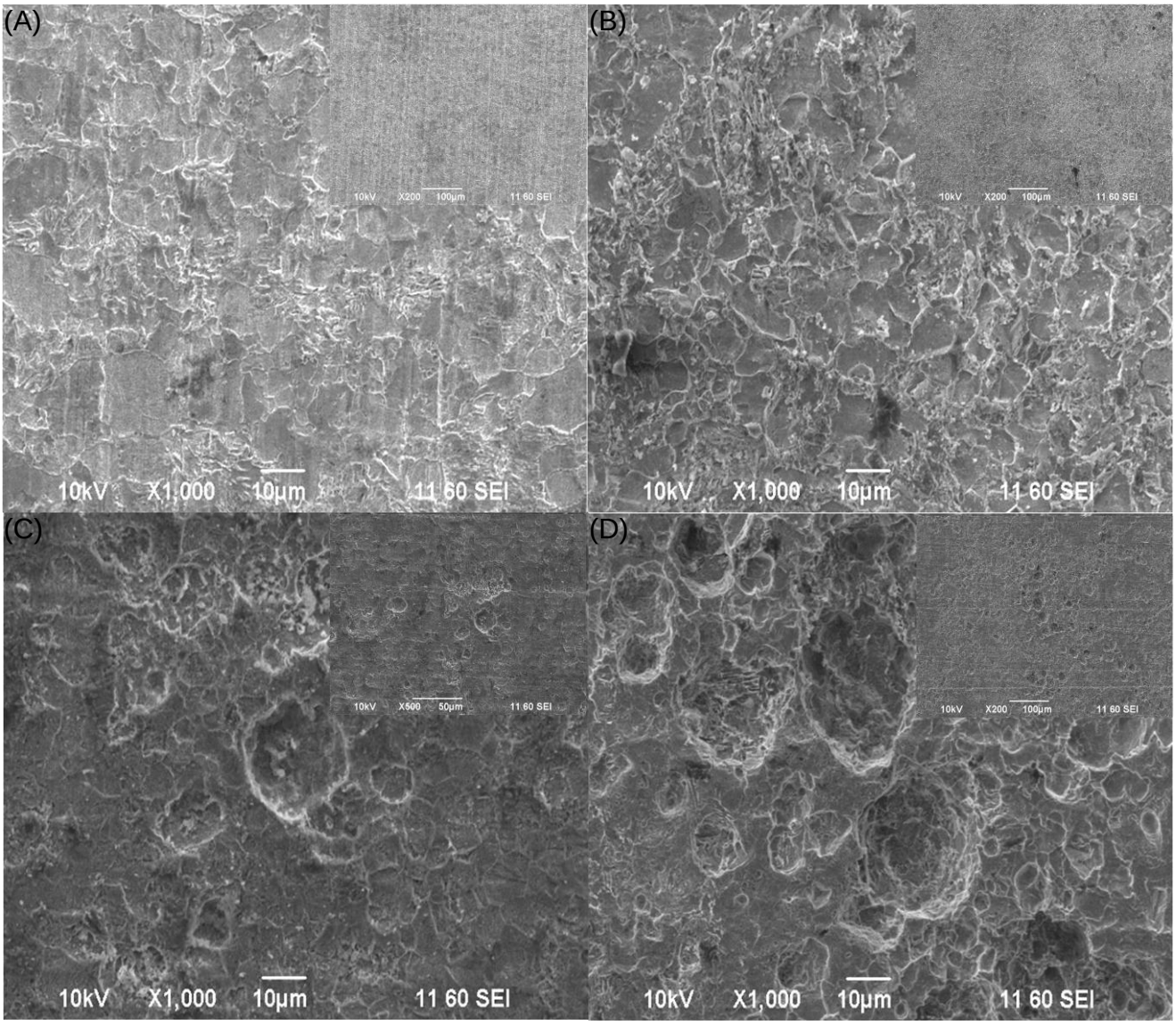

Figure 2. SEM pit images of cleaned X65 coupons after hydrotest in natural seawater: (A) no spiking at 37ºC after 90 days, (B) No SRB spiking at 22ºC after 90 days, (C) spiked with 10^6 cells/ml SRB at 37ºC after 90 days, (D) spiked with 10^6 cells/ml SRB at 22ºC after 90 days. The smaller inserted SEM images inside (A, B, D) all have a scale bar of 100 microns. For (C), it is 50 microns.

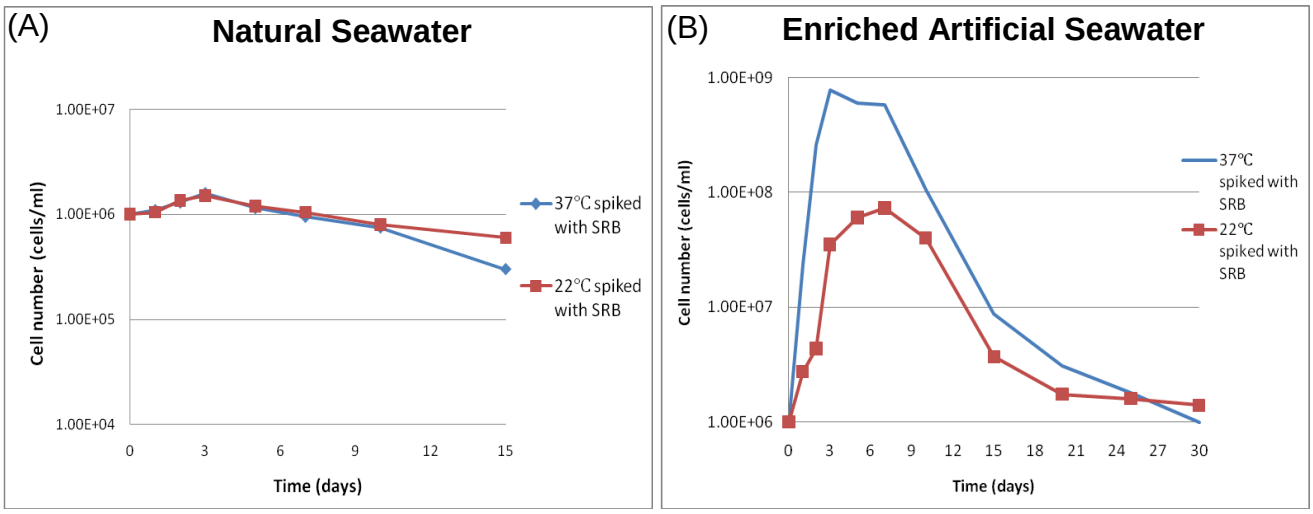

Figure 3. Planktonic cell counts in natural seawater spiked with 10^6 cells/ml SRB (A), and in enriched artificial seawater spiked with 10^6 cells/ml SRB (B).

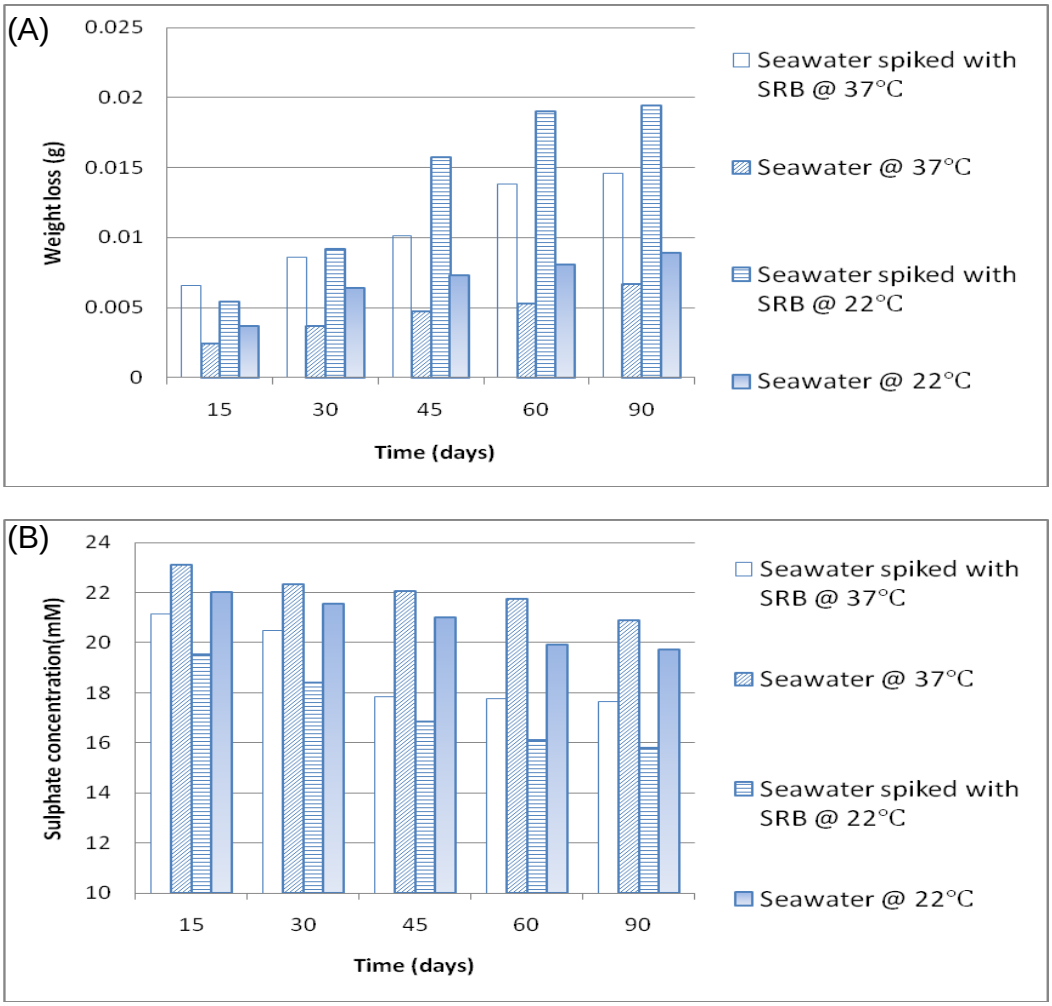

Figure 4. (A) Weight loss data for 90-day coupons, and (B) residual sulfate concentration after 90 days.

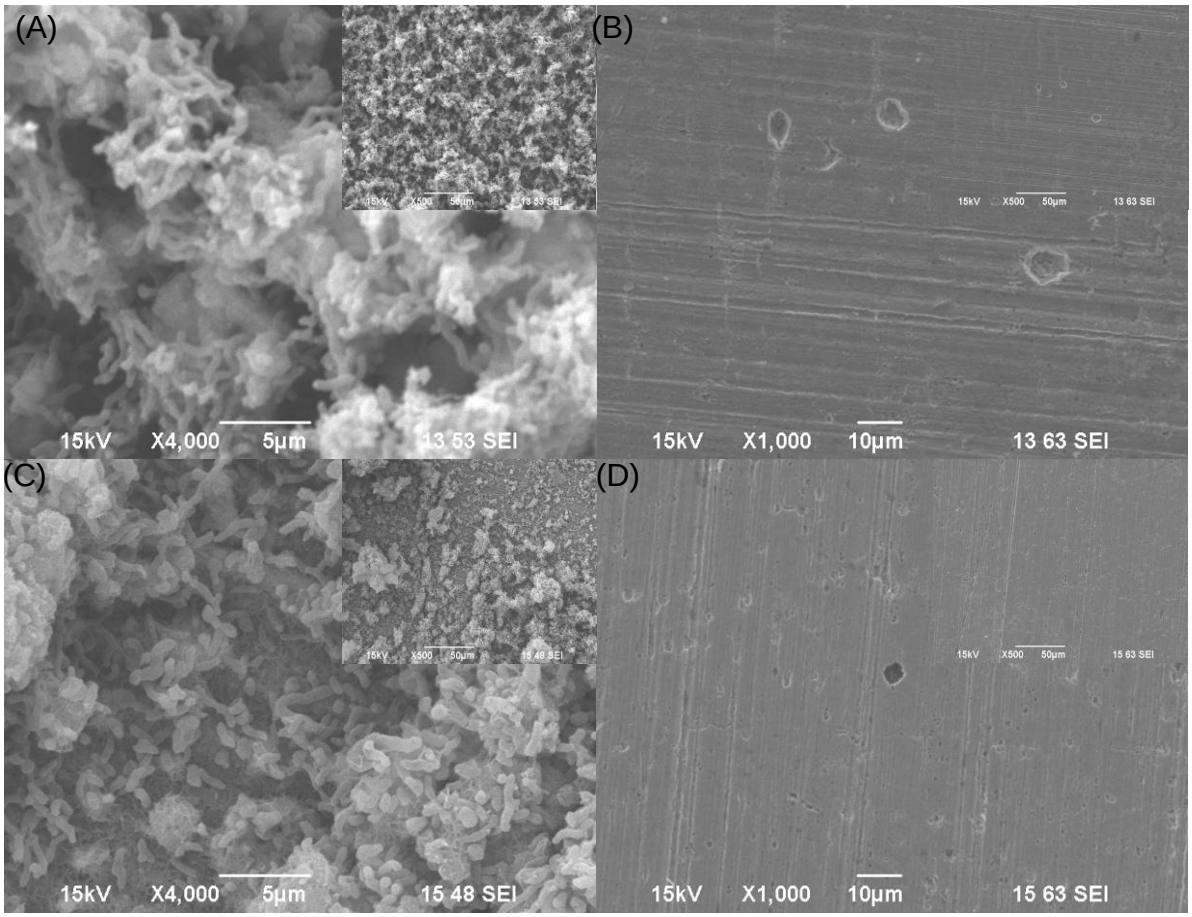

Figure 5. SEM biofilm images and pit images (after biofilm removal) on coupons. Coupons were from 30-day simulated hydrotest using enriched artificial seawater spiked with 10^6 cells/ml SRB at 22ºC (A and B) and 37ºC (C and D).

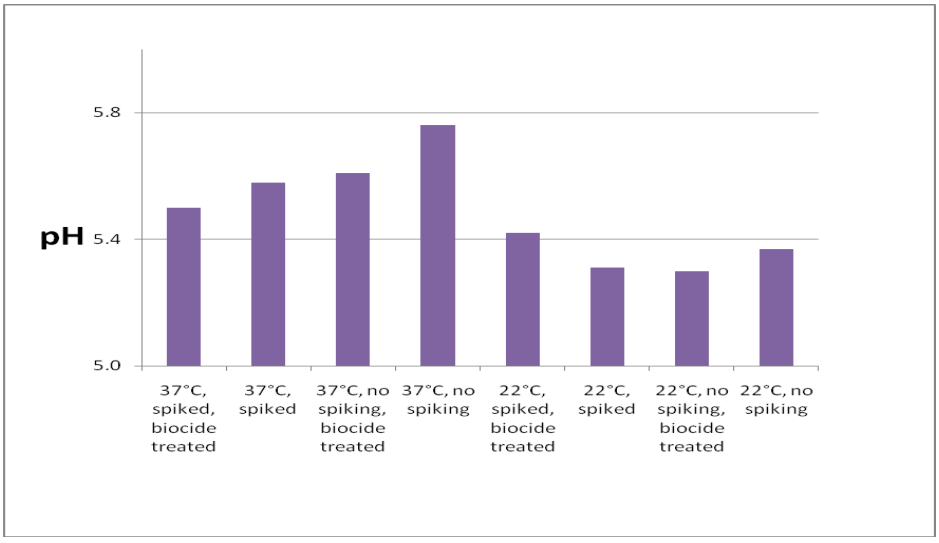

Figure 6. Water phase pH values for vials after 15 days with X65 coupons in a simulated pipeline fluid (oil mixed with natural seawater) saturated with 1 bar CO_2 in the headspace.

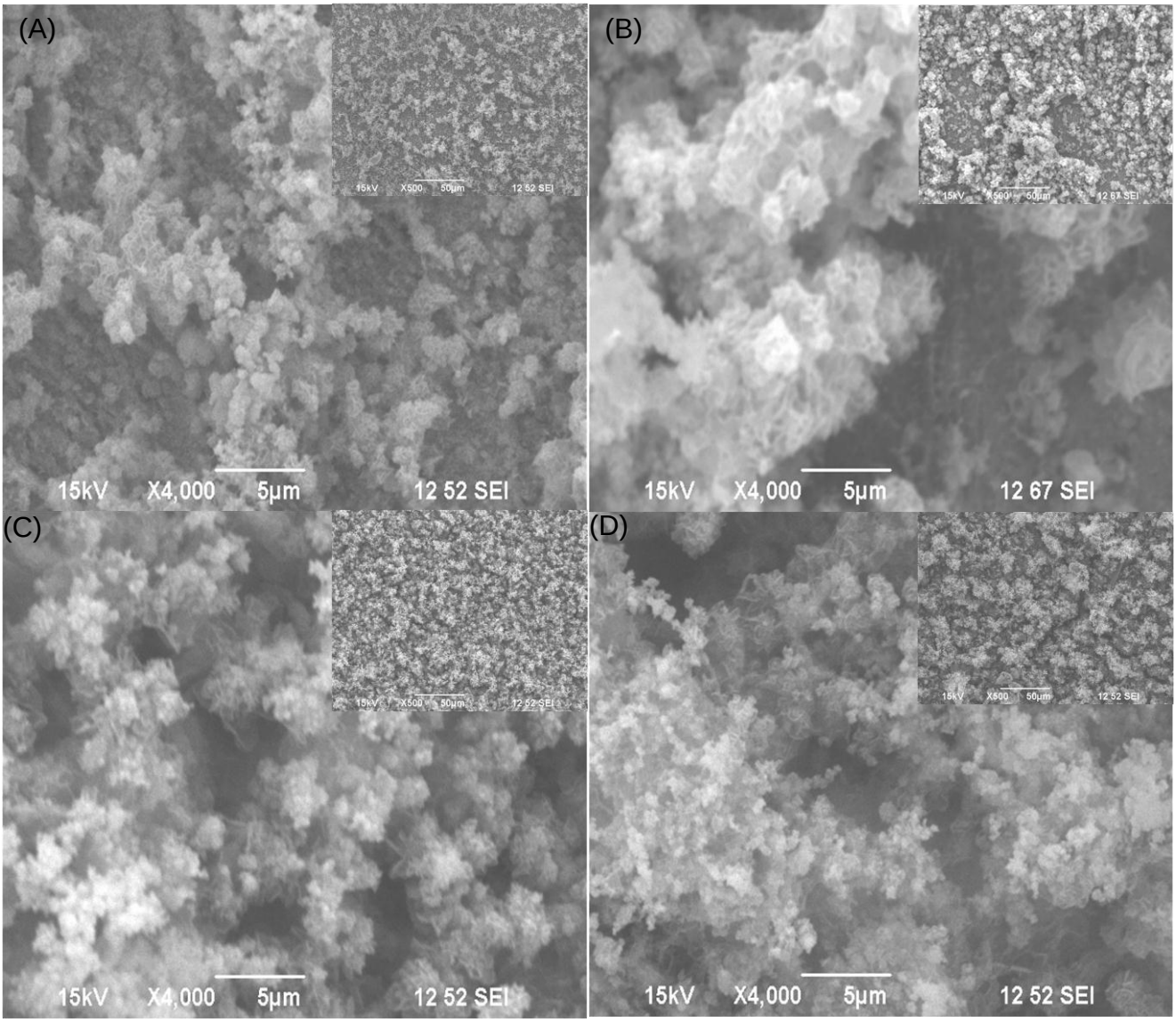

Figure 7. SEM images of X65 coupon surfaces before acid cleaning. Coupons were exposed for 15 days to a simulated pipeline fluid (oil mixed with natural seawater) saturated with 1 bar CO_2 in the headspace. Prior to the exposure, coupons were obtained from: (A) simulated hydrotest at 22ºC for 30 days in natural seawater spiked with 10^6 cells/ml SRB without biocide treatment, (B) same as (A) but with 300 ppm THPS treatment for 30 min, (C) same as in (A) but at 37ºC, and (D) same as in (B) but at 37ºC.

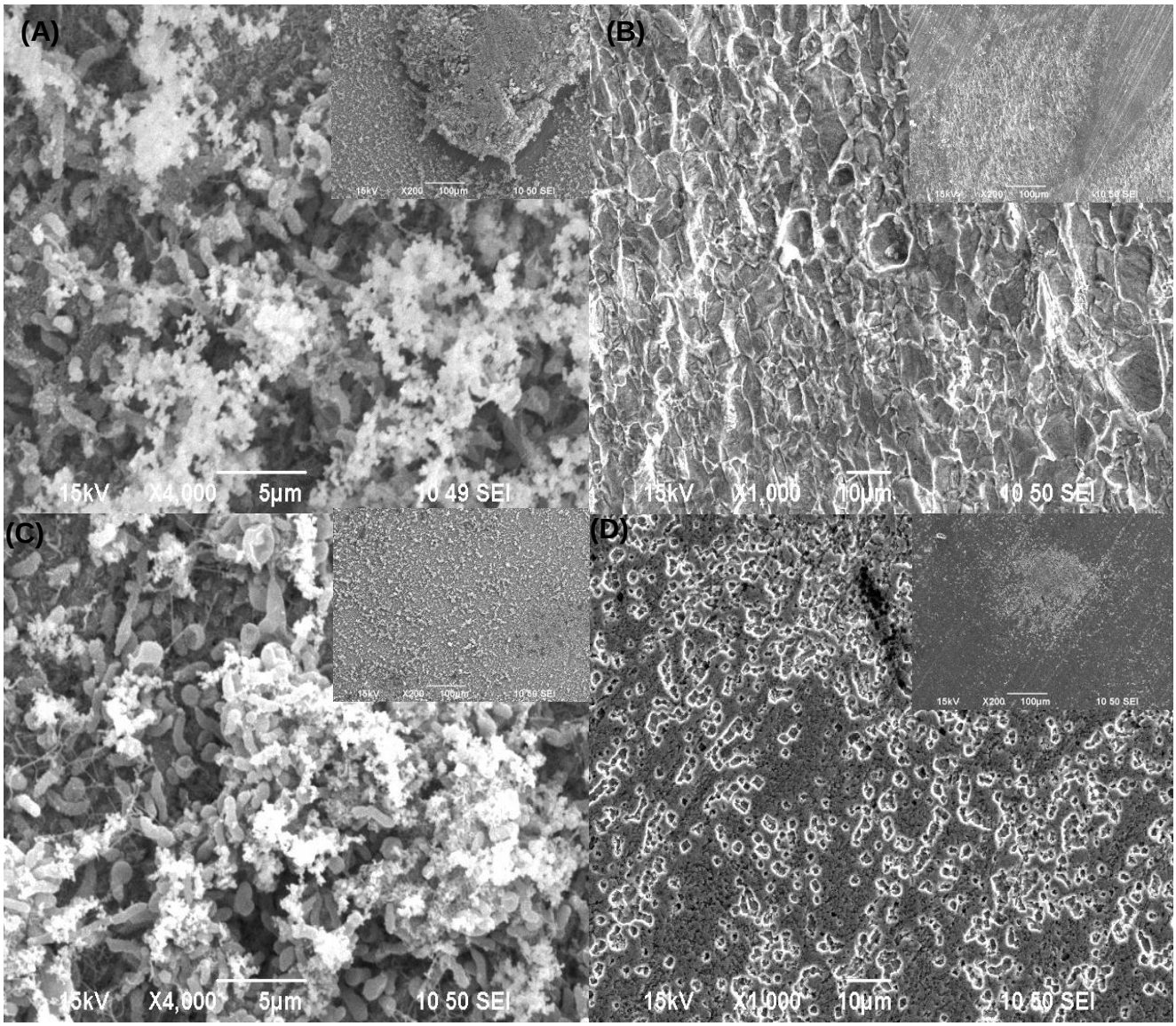

Figure 8. SEM images of X65 coupon surfaces before and after acid cleaning for biofilm (A, C) and pit (B, D) observations, respectively. Coupons were exposed for 15 days to a simulated pipeline fluid (oil mixed with simulated formation water) saturated with 1 bar CO_2 in the headspace. Prior to the exposure, coupons were obtained from simulated hydrotest either at 22ºC (A, B) or at 37°C (C, D) for 15 days in enriched artificial seawater spiked with 10^6 cells/ml SRB without biocide treatment.

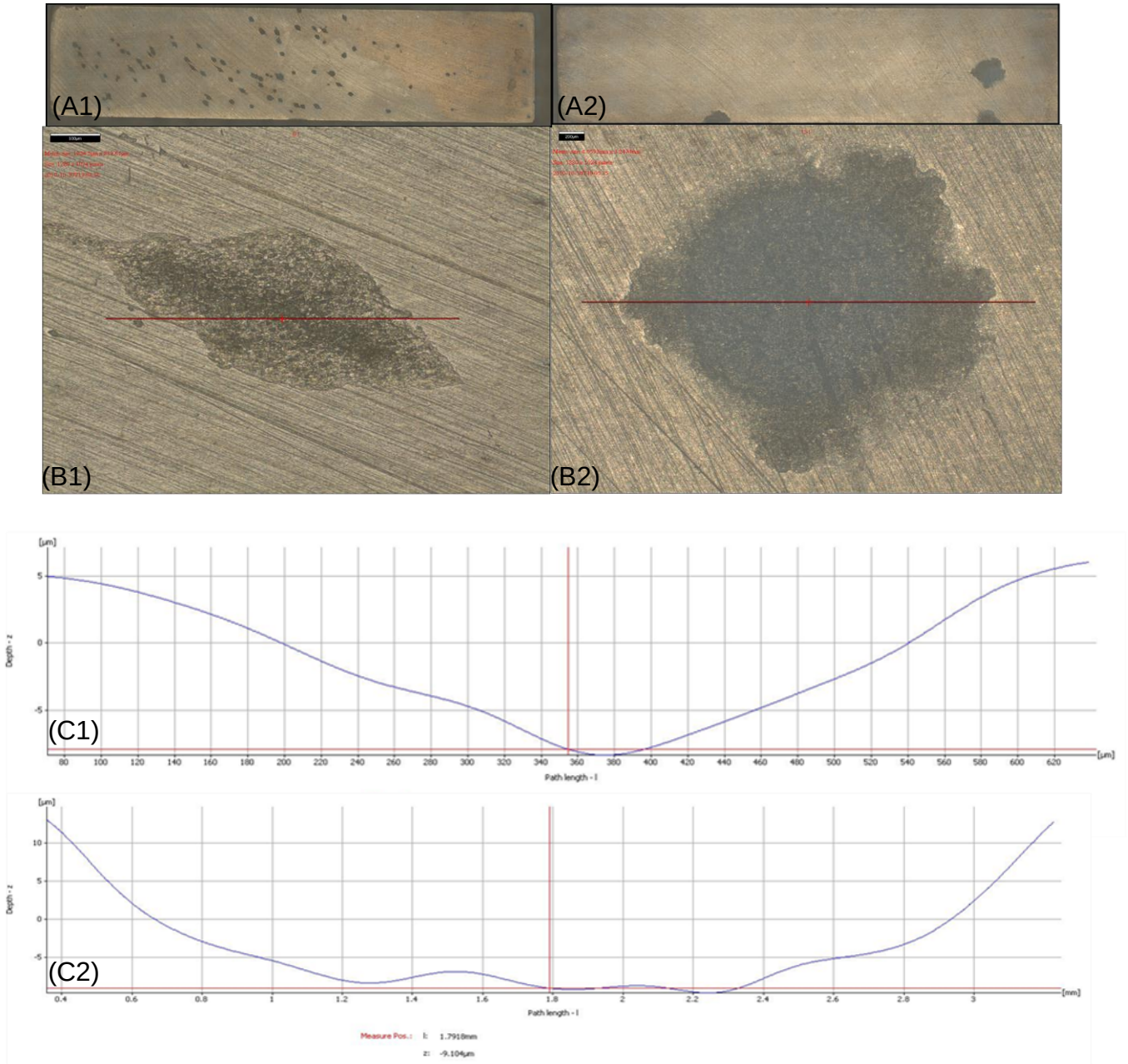

Figure 9. Profilometer analysis of a coupon surface: (A1) Overview, (B1) a selected spot; (C1) depth profile of the spot. Coupon 1 (corresponding to A1, B1, C1) was the same as the one used for Figure 8B. Profilometer analysis of a coupon surface: (A2) Overview, (B2) a selected spot; (C2) depth profile of the spot. Coupon 2 was the same as the one used for Figure 8D.