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PROOF
RESEARCH PAPER

A Comprehensive Assessment of Dissolved Oxygen Fluctuations and Biocorrosion Trends in the Sea of Marmara

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Abstract

Izmit Bay (IZ17) and Cınarcık Basin (45C) are the important points where low dissolved oxygen (DO) is observed in the Sea of Marmara. Investigating oxygen levels is essential regarding the sustainability of marine environments and evaluating possible microbiologically influenced corrosion (MIC) risks. Sulfate-reducing bacteria (SRB) play a pivotal role in the biogeochemical cycle, and they are the most aggressive group for MIC. In this study, suboxic and anoxic conditions in the Sea of Marmara were evaluated for the first time with SRB and other heterotrophic bacteria. The physicochemical parameters of seawater, nutrient analysis, hydrogen sulfide (H_2S), and sulfate measurements were performed. Total SRB and aerobic heterotrophic bacteria (AHB) cells were determined along the water column. Also, the MIC tests were performed. Results showed that the corrosion rate of Al7075 alloy was increased by SRB biofilm in real seawater. Also, suboxic conditions were observed in the bottom waters of IZ17 and 45C stations and the transition zone from suboxic to anoxic conditions at deeper depths was detected in IZ17. It indicated that the suboxic and anoxic conditions exhibit a variable profile in the Sea of Marmara. This study highlights the supportive role of examining microorganisms to better understand the biogeochemical cycles.

Introduction

Türkiye is surrounded by seas on three sides, which exhibit considerable variation in their biogeochemical properties, ecosystems, and hydrodynamics (Yilmaz, 2002). The Sea of Marmara, an inland sea, is the sole sea located entirely within the borders of Turkey (Erturac, 2002). The Sea of Marmara, which forms the Turkish Straits System (TSS), along with the Istanbul and Çanakkale Straits, has a surface area of 11,500 km², a volume of 3,378 km³, and a coastline of 972 km (Besiktepe et al., 1994; Polat et al., 1997; Ozturk, 2002). The Sea of Marmara has a two-layered water system. The upper layer constitutes low salinity waters originating from the Black Sea (18‰) entering from the Istanbul Strait, and the salty waters originating from the Mediterranean (38‰) enter from the Çanakkale Strait and, there is a sharp density interface between these two different water masses at a depth of about 25 m (Besiktepe et al., 1994). Generally, in this system, the residence time of the upper layer water is 4-5 months, and the residence time of the lower layer water is 6-7 years. (Besiktepe et al. 1993; Besiktepe et al., 1994; Besiktepe 2003). The upper layer of Sea of Marmara is subject to wind-driven turbulence. Therefore, the wind mixing can influence the overall circulation patterns in the Sea of Marmara (Besiktepe et al., 1994). Ozsoy et al. (2015) reported that the wind mixing homogenizes the water properties (like temperature, salinity, and nutrient levels), in the upper layer of the Sea of Marmara, although becoming re-stratified in the spring months (Ozsoy et al., 2015).

Additionally, its location renders it significant in terms of its impact on numerous ecosystems and human health (Tasdemir, 2002). Approximately 20% of the population of Turkey is concentrated in the Marmara region, with 87% of the region's population residing in the coastal zone of the Sea of Marmara. Industrial activities exert a considerable environmental impact on the Sea of Marmara (Tugrul & Polat, 1995; Karabayir et al., 2020). Moreover, the influx of terrestrial matter into the Sea of Marmara (Algan et al., 1999; Balkıs & Cagatay, 2001; Yasar et al., 2001; Tolun et al., 2001; 2002; Algan et al., 2004; Vidal et al., 2010; Mulayim et al., 2011; Pitta et al., 2019) the proliferation of marine traffic, and the overfishing of certain species have collectively undermined the equilibrium of the ecosystem, resulting in significant economic losses. Based on 2021 data, the total nitrogen and phosphate load in the Sea of Marmara was detected at around 329,387 and 78,257 tons/year, respectively (TUBITAK-MAM, 2021). Due to high levels of nitrogen loading, climate change, and other environmental factors, the mucilage problem was observed in the Sea of Marmara. The first serious mucilage problem was observed in the Sea of Marmara in 2007 (Tufekci et al., 2010; Balkis et al., 2011; Altıok et al., 2023). However, the intensity of mucilage events has increased over time, and in particular, a significant mucilage disaster occurred in the Sea of Marmara in 2021. Mucilage, similar to biofilm, impedes the essential activities of living organisms by accumulating on soft substrates (mud, sand, etc.) and rigid substrates (rocky, stony, etc.) or by covering these areas (Taş et al., 2016). This phenomenon has been observed to result in the sudden death of aquatic animals in marine environments. Furthermore, it results in a rapid depletion of the oxygen level and anoxic conditions (Yumun et al., 2023; Sarı and Karadurmus, 2024). The mucilage problem is one of the most significant ecological problems that emerged in the Sea of Marmara. This phenomenon began in January 2021 and continued until June 2021. The prolonged duration of the mucilage problem led to severe habitat loss and significant economic damage, therefore it has become a topic in global literature (Albay, 2023). Even before the mucilage phenomenon, recent studies have also demonstrated a decline in dissolved oxygen (DO) levels in the lower layers of the Sea of Marmara when compared to the previous two decades (Ediger et al., 2016). The dissolved oxygen (DO) values are notably low in the deep pits and gulfs of the Sea of Marmara, where the circulation is relatively limited (Balkis, 2003; Yucel et al., 2021). Izmit Bay (IZ17) and the Cınarcık Basin (45C) represent a significant location within the Sea of Marmara where low dissolved oxygen (DO) levels have been observed. These two stations have different hydrodynamic features. The 45C station, due to its location, has a more dynamic structure in both the upper and lower water layers. In contrast, IZ17, while highly dynamic in the upper layer, has a trapped water mass in the lower layer due to its position in the central basin of the Gulf, leading to longer renewal time (Sur, 1988; Muftuoglu, 2008). However, hydrogen sulphide (H_2S) has occasionally been detected in the lower water layer at both stations, attributed to seasonal fluctuations and low oxygen levels (Balkis, 2003; Yucel et al., 2021). Therefore, these two stations were selected to evaluate suboxic and anoxic conditions, focusing on sulfate-reducing bacteria (SRB).

In May and June 2021, water samples were collected from the northeast of the Sea of Marmara and IZ17 via the R/V ALEMDAR II, and preliminary experiments were conducted. The concentration of H_2S in IZ17, which has been monitored for an extended period and is typically found at a depth of approximately 110 m, was observed at a depth of 34 m following the formation of mucilage (R/V ALEMDAR II, 2021). The results demonstrated a decline in DO levels in the Sea of Marmara following mucilage. Furthermore, this case enables the proliferation of SRB in the Sea of Marmara.

SRB plays a pivotal role in elucidating the biogeochemical cycles that occur in marine environments. They are widely distributed in a variety of aquatic and terrestrial environments, including freshwaters, sediments, soils, salt waters, and oil fields (Kushkevych et al. 2021). They utilise a range of organic compounds (including pyruvate, sulfate, acetate, lactate, and others) and molecular hydrogen as the terminal electron acceptor (Unsal et al. 2023). They facilitate the reduction of sulfur compounds, resulting in the production of hydrogen sulfide. Sulfate is a significant anion present in seawater. The detection of sulfide (S^{2-}) and/or bisulfide (HS^-) compounds and H_2S in seawater is indicative of the presence and activities of SRB. Additionally, SRB exert a significant influence on microbiologically influenced corrosion (MIC). The global estimated cost of corrosion in 2018 was approximately $2.9 trillion, with 20% of this total attributed to MIC activities. The findings suggest that MIC represents a significant concern in marine environments, particularly in the context of oil and gas pipelines (Jia et al., 2019). Various bacteria, including acid-producing bacteria (APB), acetogenic bacteria, sulfate-reducing bacteria (SRB), and methanogens, as well as fungi and archaea, are commonly found in marine environments and are known to cause MIC. Nevertheless, the SRB is most often held responsible for MIC in anoxic environments (Gu et al., 2019).

The sulfur cycle encompasses many microbial pathways and chemical reactions. In these microbial pathways, SRB can use other elements (such as metals) as electron donors in place of organic carbon. Sulfate is employed as the terminal electron acceptor.

$$4Fe \rightarrow Fe^{2+} + 8e^- \ (E^{o\prime} = -447 mV) \quad (1)$$

$$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O \ (E^{o\prime} = -217 \text{ mV}) \quad (2)$$

The value of the standard reduction potential (at pH 7) is represented by $E^{o\prime}$. The redox reaction exhibits a positive cell potential (ΔE°'=+230 mV vs. SHE (standard hydrogen potential)), which corresponds to a negative Gibbs free energy. This suggests that SRB obtain energy via dissimilatory sulfate reduction (Gu et al., 2019). Aluminium alloys are frequently employed in a multitude of industrial contexts, primarily due to their low cost, high corrosion resistance and straightforward processing. Al7075 is a material that is widely used in the maritime industry, particularly in the construction of marine vehicles. It seems reasonable to posit that this alloy will become a favoured material in the maritime industry in the future. It is therefore evident that this work will be of crucial importance to future studies. The present study evaluated the dissolved oxygen dynamics and biocorrosion trends in the Sea of Marmara. Water samples were obtained from the IZ17 and 45C stations, and the physicochemical parameters (including pH, temperature, dissolved oxygen (DO), salinity, and total suspended solids (TSS)) of the water samples were determined. Additionally, nutrient analysis, H_2S, sulfate measurements, isolation, and enumeration of SRB and aerobic heterotrophic bacteria (AHB) were conducted. Furthermore, the corrosion behaviour of the Al7075 alloy was investigated in the presence of a SRB biofilm isolated from the Sea of Marmara, to ascertain the potential risks associated with minimum inhibitory concentration (MIC). This was achieved through the utilisation of Tafel and electrochemical impedance spectroscopy (EIS) methods. Furthermore, this study is significant in terms of evaluating and proposing solutions to potential MIC issues that may emerge due to corrosion in industrial sectors with marine environments.

Material and Methods

Sampling Area

The locations of the stations IZ17 and 45C which are selected in the Sea of Marmara are illustrated in Figure 1 and Table 1, respectively.

This study was conducted after the mucilage period on May and June 2022. Samples were obtained via the R/V ALEMDAR II with a CTD (Conductivity-Temperature-Depth) and Niskin bottles (General Oceanics Model 1010 Niskin Water Sampler, 5L) connected to the rosette system. For IZ17 and 45C, water samples were collected at depths of 75, 110, 125, 135, 151 m and 250, 500, 750, 900 m, respectively.

Physicochemical Analysis

The physicochemical parameters, including pH, temperature, dissolved oxygen (DO), salinity, and total suspended solids (TSS), were determined along the water column. The temperature and salinity were

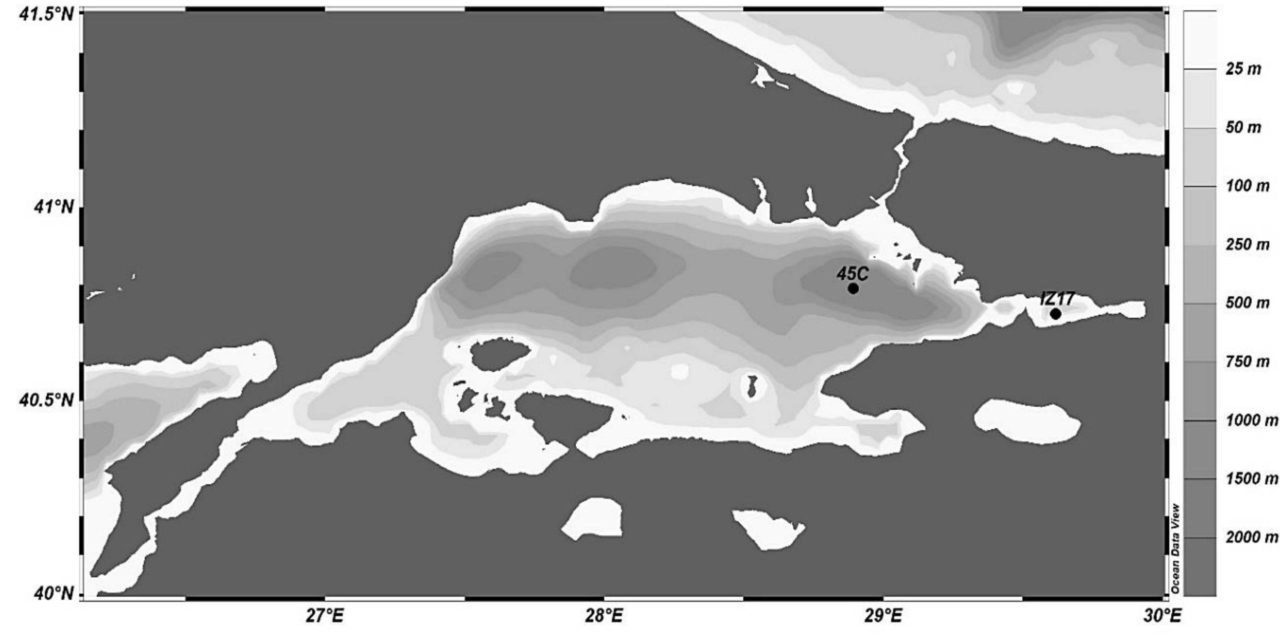

Figure 1. Map of IZ17 and 45C stations with bathymetry.

Table 1. General information for IZ17 and 45C stations

Stations	Time	Longitude	Latitude	Max Studied Depths (m)
IZ17	May 2022	29 37.01 E	40 43.30 N	151
45C	June 2022	28 53.33 E	40 47.52 N	900

measured with sensors connected to the CTD+Rosette system. The pH measurements were performed with the BENTA210 benchtop pH/mv meter. The dissolved oxygen (DO) concentration was determined by the Winkler method on board the vessel. To prevent any biological activity and contamination from the atmosphere, water samples were collected directly from the Niskin bottles (APHA, 1981). For the analysis of total suspended solids (TSS), Whatman GF/C filter papers were employed. The water samples were obtained directly from the Niskin bottles and filtered through Whatman GF/C filter papers, which were subsequently dried at 105°C for one hour. Following the final weighing of the filter papers, the TSS values were calculated in accordance with APHA, (1981) protocol.

Hydrogen Sulfide (H_2S) and Sulfate Measurements

The concentration of sulfate was determined spectrophotometrically based on precipitation with $BaCl_2$ (APHA, 1981). The total sulfur content was determined by means of an iodometric titration (Strickland and Parsons, 1972). Afterwards Subsequently, the values of H_2S were determined by measuring the pH and the dissolved sulfide concentration (Pomeroy, 1941).

Nutrient Analysis

A comprehensive nutrient analysis was conducted using an AutoAnalyzer device (BRAN+LUEBBE, Germany), which enabled the accurate determination of the following chemical constituents: ammonium (NH4+), nitrite-nitrate (NOX), silicate and orthophosphate (PO4), total phosphorus (TP), and total nitrogen (TN).

Microbiological Tests

The water samples were collected in 2 L sterile brown bottles and transported immediately to the laboratory for analysis. All samples were subjected to concentration by filtration through a sterile 142 mm diameter and 0.22 µm pore size (Sartorius) nylon membrane filter. The filtered samples were then re-suspended in 20 ml of sterile seawater using a stomacher lab blender (Interscience BagMixer) for a period of two minutes (Unsal and Caglar, 2023). The suspensions were serially diluted in a range from 10^{-1} to 10^{-7} and employed for the enumeration of AHB and SRB. From each dilution, 100 µl of sample was inoculated onto Marine Agar (Conda, Spain) plates for the enumeration of AHB. The inoculated plates were incubated at 28°C for a period of seven days. All experiments were conducted in triplicate. Following the incubation period, the number of colonies was determined by means of a colony counter (Funke Gerber, Colony Counter). The number of colonies was recorded as a colony-forming unit (CFU) in accordance with the methodology proposed by Reasoner and Geldreich (1985). SRB counts were determined using the most probable number (MPN) method in Postgate B medium (PB). The composition of the Postgate B medium (PB) is as follows (in g/L): The composition of the medium was as follows: $CaSO_4$ 1, NH_4Cl 1, KH_2PO_4 0.5, $MgSO_4.7H_2O$ 2, yeast extract 1, $C_2H_3O_2SNa$ 0.1, $C_6H_7O_6Na$ 0.1, $C_3H_5O_3Na$ 3.5, $FeSO_4.7H_2O$ 0.5, $C_2H_3NaO_2$ 2.46, resazurin 0.001 (the pH was adjusted to 7.2 by the addition of 10% NaOH) (Postgate, 1984). The MPN tubes were incubated at 37°C for a period of 90 days. Following the incubation period, the black colouration of the PB was recorded as a positive result (Postgate 1984).

Electrochemical Tests

Test coupons

Al7075 coupons were used as working electrodes. Coupons were cut in dimensions of 25×25×10 mm. All the coupons were abraded to 1200-grit, washed, dried, and kept in a desiccator until use. The copper wire was connected to each coupon and were covered with silicon leaving only a 1 cm^2 surface area. Then, sterilized under ultraviolet light (U.V) for 4 h.

Test Conditions and Biofilm test

All experiments were carried out in corrosion cell which are specialized for anaerobic environment. Platin (Pt) electrode was used as counter electrode and a saturated calomel electrode (SCE) was used as a reference electrode. To evaluate biocorrosion trends in the Sea of Marmara, SRB consortium was used (Figure 2A). Anaerobic jar (500 mL) was used for the biofilm test. The jar was filled with 300 mL Postgate's medium C (PC) with 3 mL 2-d old SRB culture. Then, Al7075 coupons were placed into the bottle and incubated at 37°C (Figure 2B). After 7 d of pre-growth, biofilms were formed on Al7075 coupon surfaces. Afterwards, the coupons were transferred to new jar containing fresh sterile natural seawater. All the tests were performed under anaerobic conditions.

Electrochemical Methods

Electrochemical measurements were performed with an electrochemical workstation (Gamry-Interface1000) in a 500 mL glass cell containing 300 mL seawater with control and test coupons. The tafel and EIS methods were used to evaluate the SRB corrosion behavior of Al7075 alloy. EIS tests were measured at OCP in the frequency from 10^5 Hz to 10^{-2} Hz with a 10 mV amplitude. Tafel curves were obtained between −250 mV and +250 mV vs OCP at a 1 mV/s scan rate. The measurement was performed after at the end of the experiment.

Results and Discussions

Physicochemical Parameters

The physicochemical parameters of water samples (such as pH, temperature, salinity, DO, and TSS) were measured (Table 2). No significant changes are observed in salinity and temperature values in both stations. Salinity values were measured as ‰38.7 and ‰38.8 in IZ17 and 45C stations, respectively. The Republic of Türkiye Ministry of Environment, Urbanisation and Climate Change (ÇŞİDB) and TUBITAK-MAM (2021) reported that the values of salinity in the lower layer of the Sea of Marmara were constant and found as ‰38.7 in spring. High salinity values in the bottom waters of the Sea of Marmara may be due to Mediterranean-origin waters that have high salinity coming with the Çanakkale Strait (Besiktepe et al., 1994; Besiktepe, 2003; Jarosz et al., 2013; Aydogdu et al., 2023). The temperature values were found as 15.9°C and 14.4°C in all depths of IZ17 and 45C, respectively (Table 2). The Sea of Marmara has a two-layer exchange flow system. Artuz et al. (2007) reported that under the intermediate layer, the bottom water temperature was 15°C (Artuz et al., 2007). For the sustainability of the marine ecosystem and life, DO levels should not decrease below 5 mg/L. However, hypoxia may occur below 2.5 mg/L in marine environments. Hypoxia is a low or depleted dissolved oxygen level and may occur naturally or with human effects in the ocean or freshwater environment (Zhang et al., 2010). A decrease in DO levels below 2 mg/L also causes suboxic conditions (Levin et al., 2009; Murray et al., 1999). DO values were measured between 0.94 mg/L and 1.90 mg/L in IZ17 (Table 2). Balkis (2003) reported that the DO level at the lower depths was below the detection limits (<0.03 mg/L). She also indicated that these results may be related to the flow of water coming from the Black Sea via the Sea of Marmara enriched with nutrients to Izmit Bay (Balkis, 2003). Also, Atabay (2012) reported that the DO values in the middle region of Izmit Bay varied between 0.3 mg/L and 1.9 mg/L (Atabay, 2012). The differences in these values also may be related to the meteorological conditions of the sampling time. Izmıt Bay is affected by weather conditions and

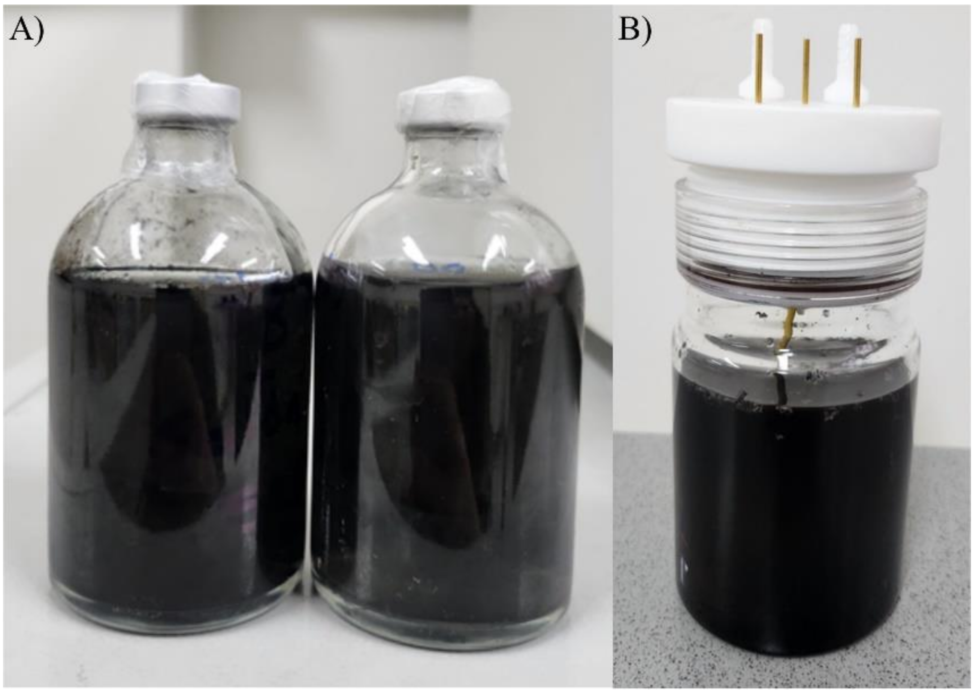

Figure 2. SRB cultures (A) isolated from the Sea of Marmara and anaerobic jar (B) for the biofilm test.

Table 2. The physicochemical parameters of the water samples obtained from IZ17 and 45C stations

Station	Depth(m)	pH	Salinity(‰)	Temperature(°C)	DO(mg/L)	TSS(mg/L)	Sulfate(g/L)	Hydrogen Sulfide (H_2S)(mg/L)
IZ17	75	8.06	38.5	15.9	1.90	28.8	2.87	*Below the detection limits
	110	8.04	38.7	15.9	1.40	29.1	2.99	*Below the detection limits
	125	8.04	38.7	15.9	0.96	29.7	2.75	*Below the detection limits
	135	8.03	38.7	15.9	1.35	27.2	3.00	0.008
	151	7.86	38.7	15.9	0.94	29.4	2.99	0.041
45C	250	8.12	38.8	14.4	1.62	26.3	2.76	*Below the detection limits
	500	8.10	38.8	14.4	2.04	12.5	3.04	*Below the detection limits
	750	8.06	38.8	14.4	1.63	21.6	2.83	*Below the detection limits
	900	8.03	38.8	14.4	1.64	23.6	3.03	*Below the detection limits

*Below the detection limits represents < 0.03 mg/L (Total sulfide concentration).

Istanbul Strait Jet. The jet negatively affects Izmıt Bay by limiting its regeneration effect (Sur, 1988; Muftuoglu, 2008; Balkis, 2003; Mutlu et al., 2024). Chiggiato et al. (2011) reported that numerous intense cyclones form in the Mediterranean Sea region annually, particularly in winter. These cyclones may cause thermal anomalies over the Aegean and Black Seas. Therefore, these cyclones also directly affect the Sea of Marmara (Chiggiato et al., 2011).

In this study, the DO values were found between 1.62 mg/L and 2.04 mg/L in 45C. Yucel et al. (2021), reported that the DO values in 45C were around 1 mg/L at the bottom depths and even <1 mg/L at some depths (Yucel et al., 2021). Researchers from Istanbul University Institute of Marine Science and Management also reported <1 mg/L during the mucilage research expedition (R/V ALEMDAR II, 2021). The most important source of oxygen for the deep basin bottom waters is the oxygen-rich Mediterranean waters coming through the deep currents of the Çanakkale Strait (Besiktepe et al., 1994; Ediger et al., 2016). Therefore, the relatively high oxygen levels observed in the deep waters during the sampling period may be associated with the influx of freshwater (ÇŞİDB, TUBİTAK-MAM 2021).

All the DO results showed that the suboxic conditions occurred in bottom waters of IZ17 and 45C. Some researchers reported that the suboxic conditions in bottom waters in 45C and IZ17 mid basin were persistent (R/V ALEMDAR II, 2021). The pH values decreased with depth in both stations, this result may be related to the low DO levels. In IZ17, the minimum pH value was found as 7.86 at 151 m (Table 2). This result can be related to the presence of H_2S at 151 m. Balkis (2003) reported that a decrease in pH was found at depths where the H_2S was detected in IZ17 (Balkis, 2003). The minimum and maximum values of TSS were detected as 27.2 mg/L (at 135 m) and 29.7 mg/L (at 125 m) in IZ17, respectively. For 45C, found as 12.5 mg/L (at 500 m) and 26.3 mg/L (at 250 m) (Table 2). Also, the high TSS values were measured at which depths oxygen levels drop.

H_2S and Sulfate Measurements

Ozturk and Alkan (2021) reported the presence of H_2S in IZ17 and 45C stations in 2021 (Ozturk and Alkan, 2021). In this study, the H_2S was found at 135 and 151 m depth in IZ17 as 0.008 mg/L and 0.041 mg/L, respectively. It may indicate that anoxic conditions may occur at these depths. Also, DO data supports these findings. The amount of H_2S in IZ17 was measured to range from 0.31 mg/L to 1.07 mg/L in 2005-2006 (Sur et al., 2006). Balkis (2003) reported that the amount of H_2S was found as 1.25 mg/L in IZ17 after the earthquake in 1999 (Balkis, 2003). After mucilage, the H_2S in IZ17 was found around 34 m water depth and up to 2 mg/L (R/V ALEMDAR II,2021). Yucel et al. (2021) also reported that the H_2S was detected in bottom waters in 45C (Yucel et al., 2021). However, in this study, the amount of H_2S was detected as quite low comparing recent years. The reason for that could be the oxygen-rich Mediterranean waters coming through the Çanakkale Strait (Besiktepe et al., 1994; Ediger et al., 2016). During the mucilage period in 2021, water samples were collected from the northeast of the Sea of Marmara and IZ17 via the R/V ALEMDAR II, and preliminary experiments were carried out. The concentration of H_2S in IZ17 was observed at a depth of 34 m following the formation of mucilage (R/V ALEMDAR II, 2021). The results showed that a significant decline in DO levels in the Sea of Marmara during the mucilage period. However, this study was conducted after the mucilage period on May and June 2022. Therefore, based on the results in DO and dissolved H_2S values during and after mucilage period, could indicate that the Marmara waters renew itself despite all adverse conditions (R/V ALEMDAR II, 2021). Changes in DO and dissolved H_2S values during and after mucilage period could indicate that the Marmara waters renew itself despite all adverse conditions (R/V ALEMDAR II, 2021). The values of sulfate were determined between 2.75 g/L and 3.00 g/L in IZ17. Similar values were reported by researchers (Sur et al., 2006). In 45C, no remarkable change is observed in sulfate values depending on the depths. It is known that the sulfate concentration in seawater should be globally homogeneous, reflecting the overall global redox state of the atmosphere and ocean system (Zhu et al., 2021). Oxidation reduction (redox) potential, one of the essential parameters that plays a critical role in understanding the suboxic and anoxic levels. In redox potential, sequentially, oxygen is reduced first, followed by nitrate then oxidized manganese, ferric iron and sulfate are reduced (Xu et al., 2018).

Nutrient Analysis

The depth profiles of nutrients for IZ17 were given in Figure 3. Temporal fluctuations were observed in nutrient concentrations (Figure 3). The maximum value of TP, NO_x NH_4^+ silicate, PO_4 and TN was found as 0.69 µM at 125 m and 151 m, 7.29 µM at 135 m, 3.67 µM at 125 m, 29.55 µM at 125 m, 1.25 µM at 110 m and 55.02 µM at 125 m, respectively. For 45C, the maximum value of TP, NO_x NH_4^+ silicate, PO_4 and TN was found as 0.88 µM at 500 m, 3.75 µM at 900 m, 2.97 µM at 500 m, 36.49 µM at 900 m, 0.68 µM at 500 m and 24.55 µM at 500 m, respectively (Figure 4). The maximum TP, NH_4^+, silicate and TN values were determined where DO level is low. The maximum NO_x and PO_4 values were lower in 45C compared to IZ17. Aslan Yilmaz (2008) also reported that the NO_x and PO_4 values which reach the highest level where oxygen level is low, tend to decrease in bottom waters below 500 m. She reported the values range from 0.7-1.1, 7.8-10.7, and 32-39 µM for PO_4, NO_x, and reactive silicate, respectively (Aslan Yilmaz, 2008). These results showed that the dynamics of nutrients have been influenced by DO levels in the Sea of Marmara.

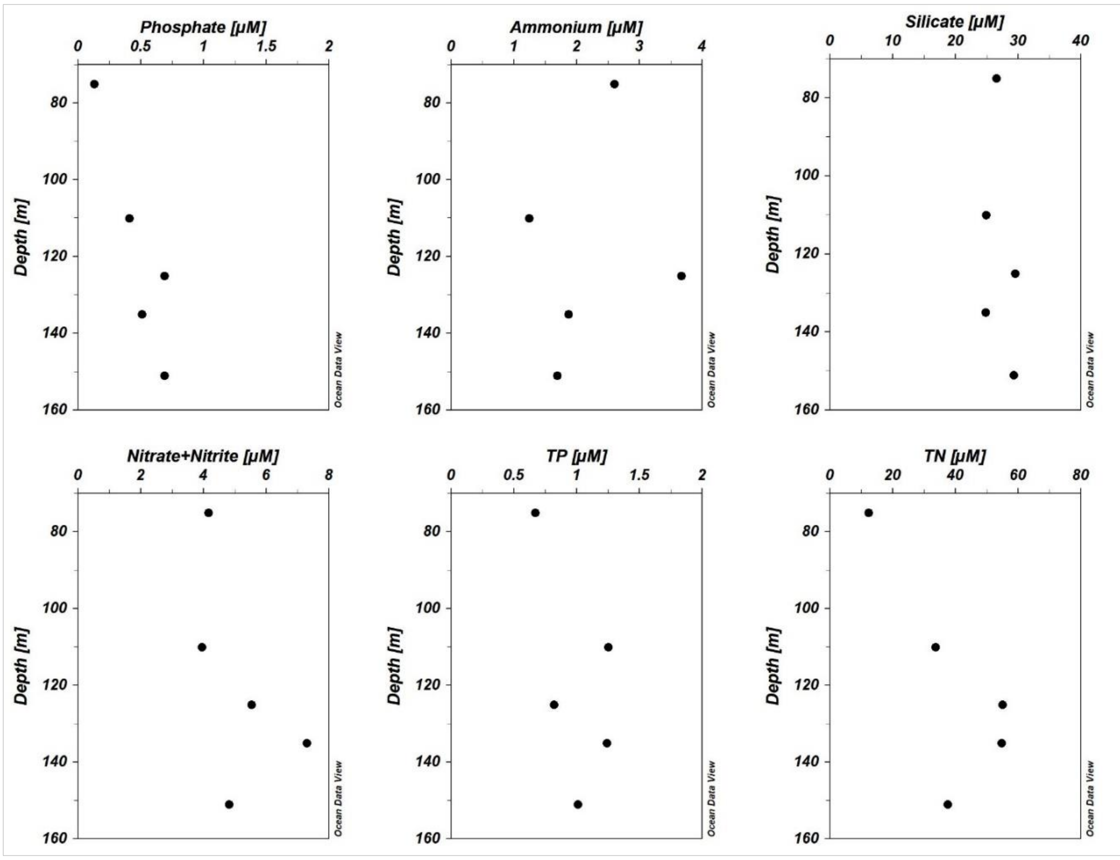

Figure 3. Depth profiles for major nutrients in IZ17.

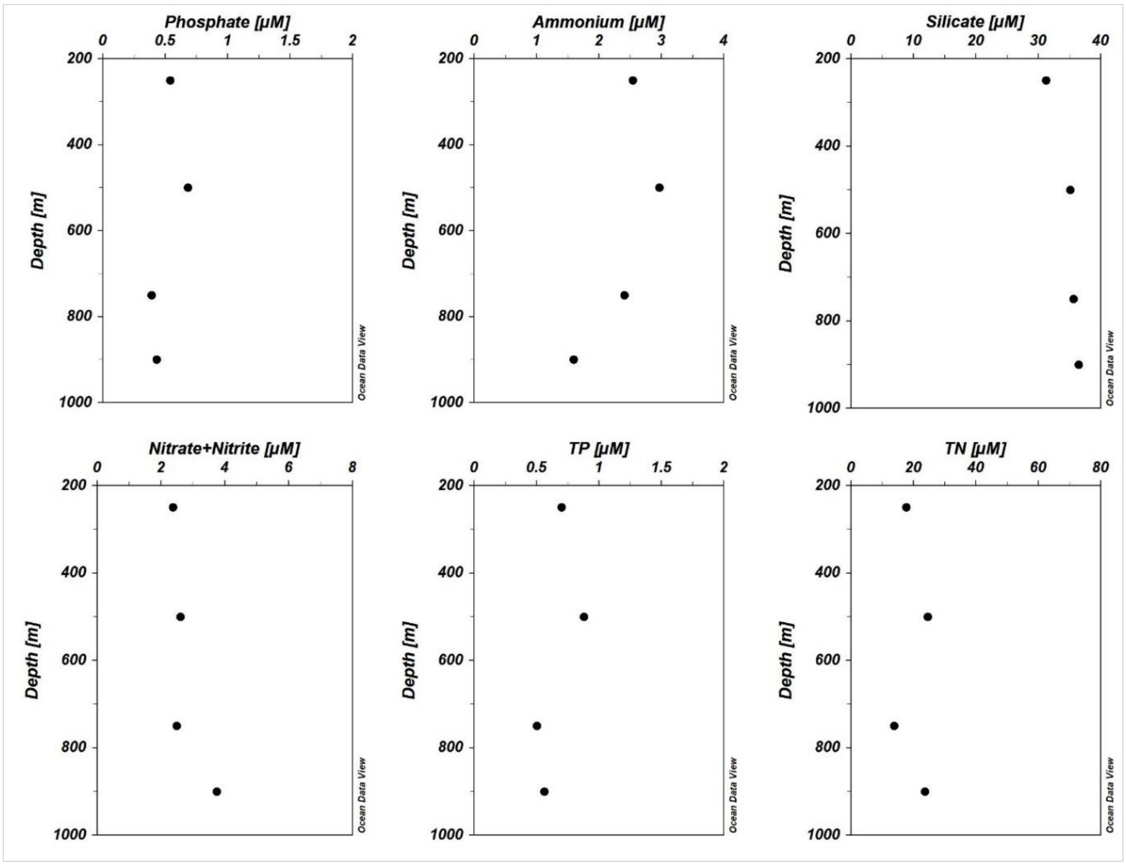

Figure 4. Depth profiles for major nutrients in 45C.

Microbiological Analysis

Enumeration of AHB and SRB Cells

The total number of AHB and SRB cells depending on depths was given in Figure 5. In IZ17, the number of AHB cells increased up to 135 m, then decreased and increased again at 151 m. The maximum and minimum AHB cells were found as 78378 cfu/ml at 135 m and 385 cfu/ml at 125 m, respectively. In 45C, the AHB cells decreased with depth, the maximum and minimum AHB cells were detected as 63000 cfu/ml at 250 m and 9150 cfu/ml at 900 m, respectively (Figure 5B). The total number of AHB cells at studied depths were found higher in 45C compared to IZ17. Although 45C is deeper than IZ17, the detection of high AHB cells, indicates that the abundance of AHB bacteria is affected by deep discharges in the Black Sea strait's impact area. Aslan Yilmaz (2008) also reported that the number of bacteria increases on the coasts and at the discharge points, and in some periods, depending on the hydrodynamic conditions, the number of bacteria also increases in the deep parts (Aslan Yilmaz, 2008). In IZ17, the maximum and minimum SRB cells, were found as 13300 cell/ml at 135 m and 20 cell/ml at 125 m, respectively. Barton and Hamilton (2007) reported that in saltwater environments, SRB cells were usually found low, compare to total bacterial population (Barton and Hamilton, 2007). In 45C, the maximum and minimum SRB cells, were found as 85000 cell/ml at 750 m and 3000 cell/ml at 250 m. The total number of SRB cells increased with depth in 45C. An increase in SRB cells at the depths where the NOx and DO values are low, may indicate that the sulfate reduction begins. The detection of the presence of SRB, especially at the points where suboxic and anoxic conditions begin, is very important in terms of understanding the functioning of the ecosystem in these regions, evaluating the formation of H_2S and understanding the biogeochemical cycles (Yucel et al. 2021). Also, the H_2S was detected in these depths. The presence of H_2S in IZ17 indicates that the nitrate ions and even partially sulfate ions are used as electron acceptors in organic matter degradation. Thus, the denitrification occurs followed sulfate reduction comes (Millero, 2000). However, H_2S was not observed in bottom waters in 45C. This indicates that there is not

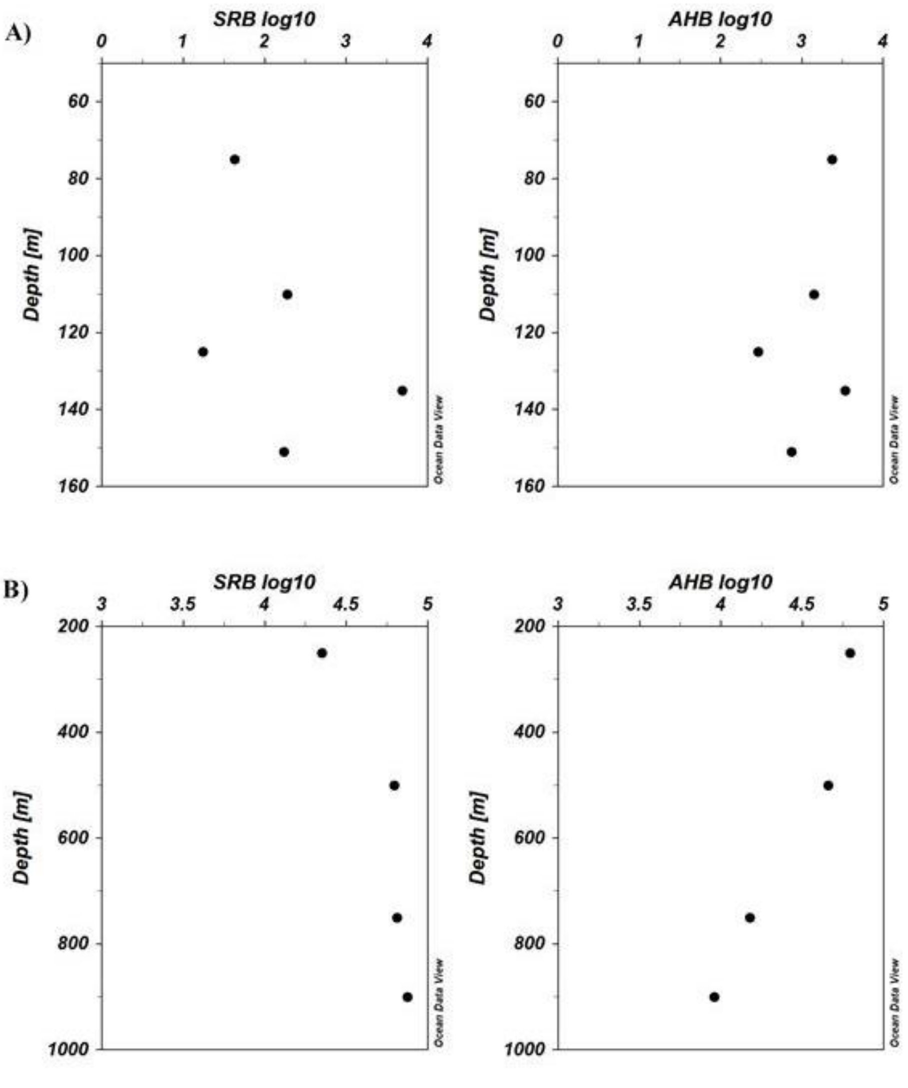

Figure 5. Depths profile for the total number of SRB and AHB in A) IZ17 and B) 45C.

always a relationship between the number of SRB cells and their activities. Indeed, Santegoeds et al. (1998) also reported similar results in their study on the population dynamics of SRB (Santegoeds et al. 1998). The total number of SRB cells were found more at upper depths in IZ17 compared to 45C. Generally, SRB could be found in deeper waters due to anoxic conditions. However, SRB can also be found in surface waters where low redox conditions observed. Methanogenic archaea, and SRB are commonly present together in high-sulfate and low-sulfate environments, but they usually do not compete each other to degrade the organic matter. Methanogens usually degrade non-competitive compounds (Kushkevych et al. 2021). In freshwater environments, sulfate reduction does still occur, even if available sulfate is usually limited, however methanogenesis dominates in this process (Purdy et al. 2003).

Electrochemical Analysis

Electrochemical parameters (corrosion potential (E_{corr}), corrosion current density (i_{corr}), anodic and cathodic Tafel slope values (β_α and β_c)) of Al7075 coupons are shown in Table 3. The E_{corr} value of the control coupon was found −0.902 mV/SCE. After 7-d of incubation, the E_{corr} value of test coupon shifted to more positive potential (−0.672 mV/SCE) (Figure 6, Table 3). At the end of the 7-d incubation period, the i_{corr} value for test coupon was found as 10.3 µA/cm^2. The i_{corr} value of test coupon was approximately ten times higher than the control coupon (1.7 µA/cm^2) (Figure 6, Table 3). It indicated that SRB biofilm accelerated the corrosion rate of Al7075 coupon.

Nyquist, Bode, and phase-frequency curves of Al7075 coupons are given in Figure 7. EIS data showed that the coupons with biofilm had significantly promoted the corrosion rate of Al7075 coupon (Figure 7). Feng et al. (2022) also reported similar results. They found that the impedance resistance of Al7075 was significantly reduced by SRB biofilm in real seawater (Fang et al., 2022). The EIS data also were modeled with equivalent electrical circuits (Figure 8). The fitting parameters are given in Table 4. Figure 8A shows the circuit for the control coupon. The constant phase element (CPE) was used to replace the ideal capacitor (Unsal et al., 2019).

$$Z_{CPE} = Y_0^{-1} (j\omega)^{-n} \quad (3)$$

In Figure 8A, the circuit includes (1): R_s as solution resistance, (2) charge transfer resistance R_{ct} and CPE_1 for the formation of a heterogeneous layer containing mainly corrosion products, (3) the parallel combination of R_f as and CPE_2 for the oxide film layer on the Al7075 coupon surface. In Figure 8B the circuit includes: (1) R_s, (2) the parallel combination of R_{ct} and CPE_1 for the double layer capacitance and CPE_2 for the biofilm resistance (R_b) with a infinite Warburg element (W). In the presence of SRB biofilm, the Warburg element was observed. It represents the diffusion process. Also, EIS data confirmed that SRB biofilm accelerated the MIC of Al7075 alloy because it led to decreased R_{ct} in Table 4.

Table 3. Electrochemical parameters derived from the Tafel analysis of control and test coupons

Parameter	Control	Test
E_{corr} (mV) vs. SCE	-0.902	-0.672
I_{corr} (µA/cm^2)	1.7	10.3
β_a (mV/dec)	0.73	0.26
β_c (mV/dec)	0.18	0.95

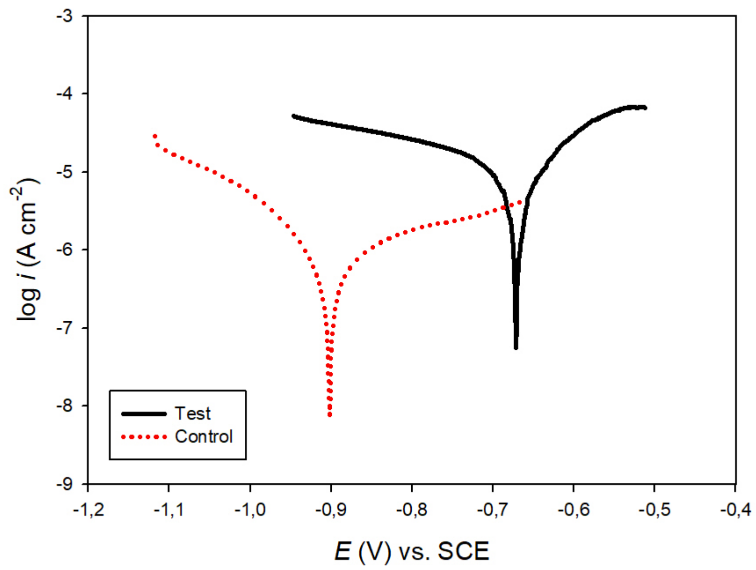

Figure 6. Tafel curves for control and test coupons.

Conclusion

The eastern basin of the Marmara Sea is the most densely populated urban and industrial area. In this region, terrestrial pressure is quite high. Suboxic and anoxic conditions occur in this eastern basin. Suboxic and anoxic conditions are dynamic in 45C. In contrast, at IZ17, there are permanent anoxic conditions in the bottom water. Therefore, these areas are important for understanding SRB's role better. 45C, the deepest pit of the eastern basin, and IZ17, where terrestrial pressures are most intense and the most polluted gulf of the central basin, therefore these areas were selected. Moreover, MIC problems in the marine environment are important nowadays, especially in maritime traffic, ships, and pipeline systems. IZ17 station is located where ports and industrial activities are intense, so it is important to evaluate it in this context. In recent studies, suboxic and anoxic conditions have been determined only by physicochemical parameters. This study is the first time suboxic and anoxic conditions were evaluated with SRB and other heterotrophic bacteria. Also, MIC in the marine environment has rapidly become serious issue, particularly in maritime traffic, ships, and pipeline systems. In this study, for the first time, it was found that the SRB biofilm isolated from the stations accelerated the corrosion rate of the Al7075 alloy in real seawater. This provides significant insights into the sustainability of marine ecosystems and future MIC challenges. It also indicates the need to consider the current and future MIC challenges in the Sea of Marmara.

In conclusion, the suboxic conditions were observed in the bottom waters in IZ17 and 45C after the mucilage period in the Sea of Marmara in 2022. Also, the transition zone from suboxic to anoxic conditions were detected in IZ17. It indicates that the suboxic and anoxic conditions exhibit a variable profile in the Sea of Marmara, especially in the mentioned stations. This study emphasizes the importance of investigating SRB and other bacteria to understand biogeochemical cycles better. It serves as a supporting parameter such as pH and reduction potential (EH) parameters. Future studies should be designed to include long-term, seasonal, and more frequent sampling to thoroughly understand the dynamics in these areas and develop an action plan accordingly.

Ethical Statement

Ethical Approval is not required.

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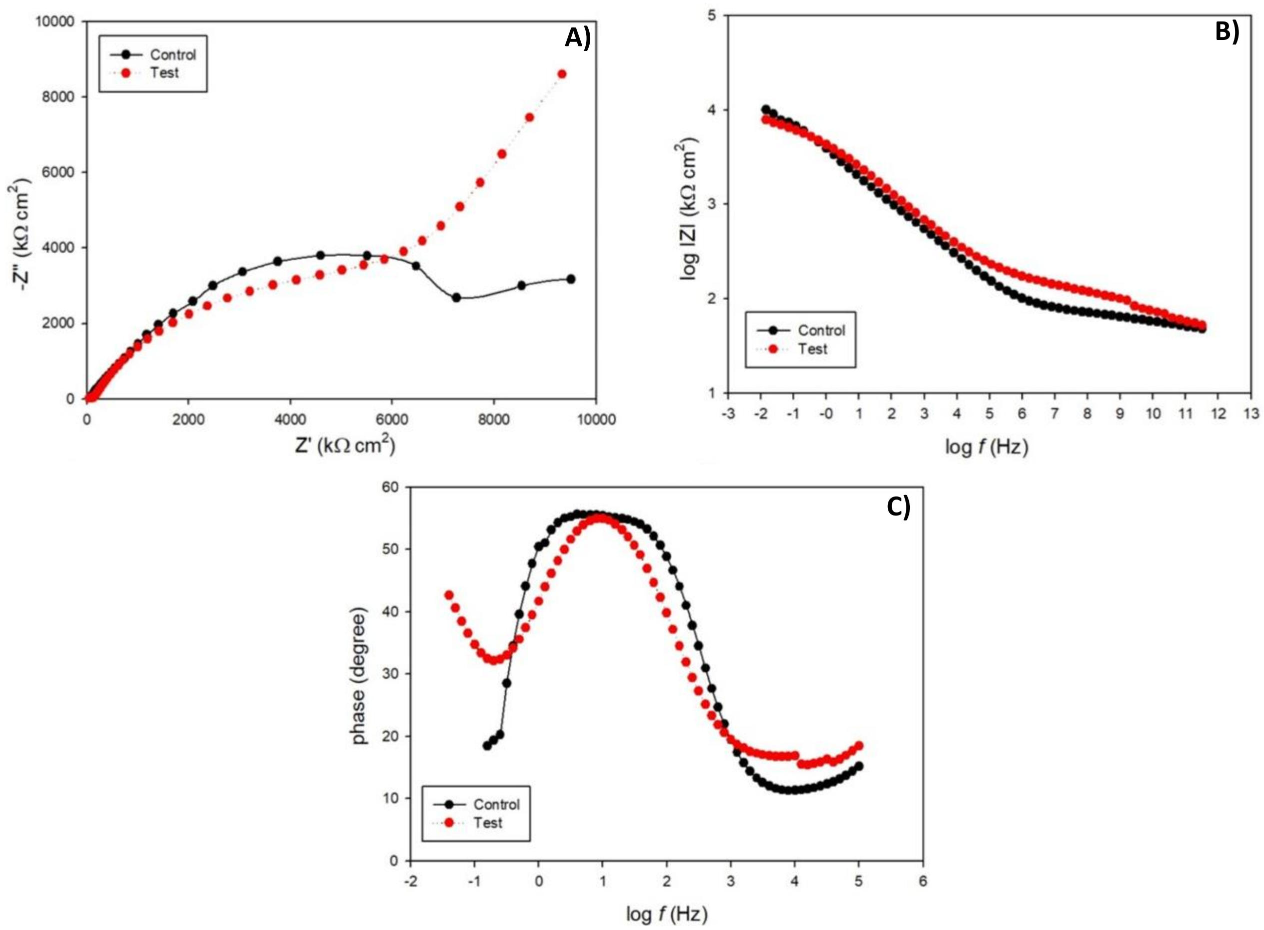

Figure 7. Nyquist (A), Bode (B) and phase-frequency (C) plots for control and test coupons.

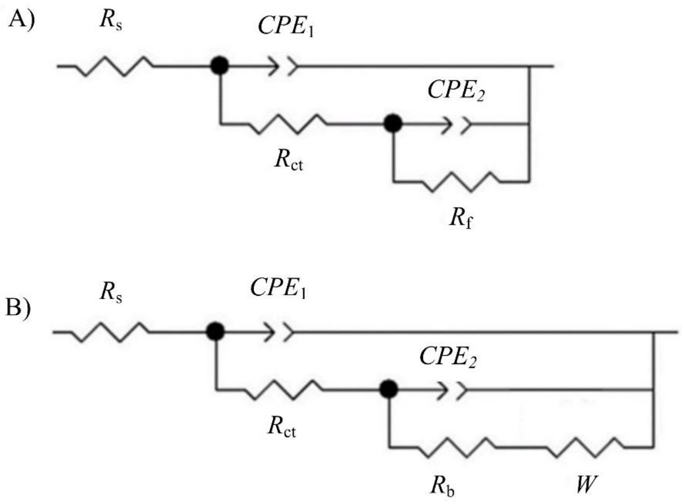

Figure 8. Circuit models used for fitting EIS data: (A) control (B) test (with biofilm) coupons.

Table 4. Electrochemical parameters from fitting EIS data

Medium	R_s(Ω cm^2)	Y_2(Ω^{-1}cm^{-2} s^n)	n_2	R_b(kΩ cm^2)	Y_1(Ω^{-1}cm^{-2} s^n)	W(Ω cm^2)	n_1	R_{ct}(kΩ cm^2)
Control	61.6	0.284	0.84	1186 (R_f)	0.262	-	0.85	7313
Test	47.4	0.165	0.46	3210	0.548	0.126	0.92	5439

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Author Contribution

Seben Yucel: Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing; Tuba Unsal: Conceptualization, Funding Acquisition, Project Administration, Resources, Supervision, Writing -review and editing; Nuray Balkis Caglar: Supervision, Conceptualization, Methodology, Writing - review and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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