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Corrosion Behavior of AISI 1045 Carbon Steel in Metalworking Fluids Containing *Pseudomonas xiamenensis*

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Microbial contamination of aqueous metalworking fluids (MWFs) is of significant concern in metal process industries. In this study, a dominant *Pseudomonas* sp. strain (*P. xiamenensis*) was isolated from waste metalworking fluids (WMWFs) and the biodegradation of model MWF was studied under the influence of *P. xiamenensis*. Results showed that nitric additives in MWF were severely degraded by bacteria, leading to a significant increase in the turbidity of the MWF. The occurrence of microbially influenced corrosion of AISI 1045 carbon steel in MWF containing *P. xiamenensis*, was proven by morphological analysis and electrochemical characterization. The corrosion rate of the metal in MWF in the presence of bacteria was three-fold greater than in the absence of bacteria. Electrochemical results and surface analysis elucidated that the additive film was decomposed by *P. xaimenensis* adhered to the steel surface and a biofilm, which was controlled by diffusion replacing the additive film. The biofilm was reinforced by the reaction of metabolites and Ca^{2+} and seriously localized corrosion behavior was observed in carbon steel immersed in the bio-contaminated MWF due to the microbial influence.

Keywords: metalworking fluid; biodegradation; AISI 1045 carbon steel; microbially influenced corrosion; electrochemistry

1. INTRODUCTION

Metalworking fluid (MWF) is an industrial lubricant that is widely applied in machining processes to improve tool life and attain the required surface [1]. To improve cutting speed and accuracy, the tendency to use aqueous MWFs is continually growing, as shown by the increasing production of MWFs. Data shows that approximately 38 million tons of MWFs were used worldwide in 2005, with an

increase in use of 1.2% reported in 2015 [2]. Aqueous MWFs are diluted into oil/water (O/W) emulsions at a 2-20% concentration range. Mineral oils, surfactants, corrosion inhibitors, buffer agents and other chemical additives to diluted aqueous MWFs provide excellent lubricating, cooling, cleaning and anti-corrosion functions in metal processing.

However, there is a high potential for microbial contamination of MWFs due to a lack of appropriate maintenance. Microorganisms in the diluting water, personal handling machine tools and adsorbed onto dust and aerosols, can contaminate MWFs and high microbial counts can rapidly be found in the MWF due to a lack of proper microbial control. Bakalova et al. [3] reported an aerobic bacterial count of 3.2×10^3 CFU (Colony Forming Units)/mL in MWF after one month and it has been reported that the microbial count can range from 10^4 to 10^{10} CFU/mL in MWF [4]. Various microbial species have been found in MWFs, such as *Pseudomonas sp.*, sulfate-reducing bacteria (SRB), *Staphylococcus* and *Alcaligenes* [4–7]. Furthermore, various kinds of pathogens have been isolated from MWFs such as *Klebsiella pneumonia*, *Escherichia coli*, *Legionella* sp. and *Mycobacterium immunogenum* [8–10]. Many reports have proposed that workers exposed to MWFs containing *Mycobacterium immunogenum* can suffer from hypersensitivity pneumonitis (HP), asthma and other respiratory diseases [9,11,12].

High levels of microbial contamination directly result in the failure of MWF, with microbial growth in the MWF changing its stability and leading to demulsification that may reduce tool life during the machining process [13]. The explosive growth of microorganisms may form biofilms, causing clogging of the pump, pipeline and other components of the machining system. The metabolites produced by microorganisms change the properties of MWF significantly. MacElwee et al. [14] indicated that biodegradation of the emulsifying agent was influenced by extracellular polymeric substances (EPS) from the genus *Pseudomonas*, which increased the droplet size of the emulsion. Previous research has shown that buffer agents, like monoethanolamine (MEA) and triethanolamine (TEA), are degraded by microorganism [15], leading to a decrease in the pH of MWF and causing corrosion of workpieces, cutting tools and machine tools. Zhang et al. demonstrated that the corrosion rates of WC-30Co cemented carbide increased about 10-fold in the presence of MWF containing SRB, as opposed to in the presence of fresh MWF [16].

According to the biodegradation of MWF, the anti-corrosion function was dramatically weakened in deteriorated MWF solutions [3,17–19]. Components in the MWF, like mineral oil, emulsifying agents, corrosion inhibitors and anti-foam agents, provide an abundant nutrient source for the colonization of microorganisms. Malfunction of the anticorrosion function of MWF not only resulted from the biodegradation of additives in aqueous solution but was also influenced by bacterial metabolism [20]. The corrosion of metal is a ubiquitous phenomenon during such aggressive processing scenarios. Medium carbon steel like AISI 1045 steel is a common manufacturing material in the machining process, due to its excellent mechanical properties and high availability. This carbon steel is not only manufactured into structural components but is also fabricated into machine tool accessories, such as MWF tanks, tramps and machine tool spindles. These accessories are essential parts of machine tool systems and much research has addressed the corrosion behavior and mechanisms of AISI 1045 steel in aqueous solutions, when exposed to microbial contamination. However, there are few studies focusing on microbially influenced corrosion (MIC) of AISI 1045 steel in aqueous MWF systems. The corrosion of tramps and machine tool spindles, will cause the deviation of machining accuracy, thereby forming

defects in the product. Furthermore, leakage of MWF will occur in the workshop if pitting corrosions fully penetrates through pipelines and MWF tanks. It is important to evaluate the MIC behavior of AISI 1045 steel in MWF containing single bacteriums or multiple bacteria.

In this study, a species of *Pseudomonas* was isolated from WMWF and the biodegradation of MWF was studied. The MIC of AISI 1045 in biodegraded MWF containing these bacteria was investigated through morphologic analysis and electrochemical measurement.

2. MATERIALS AND METHODS

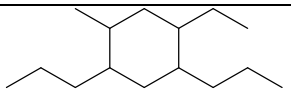
2.1 Materials

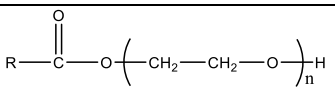
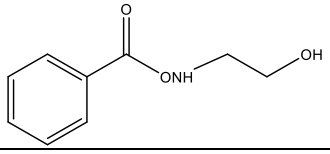
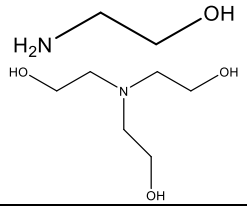
Glutaraldehyde (25% v/v), ethanol (95% v/v), glycerol, acetone (95% v/v), hydrochloric acid (36% v/v), sodium bicarbonate (> 99.5%) and agar were purchased from Sinopharm chemical reagent Co. Ltd (Shanghai, China). Acridine orange (AO) was obtained from Aladdin Co. (Shanghai, China). LB broth and 2216E culture medium were purchased from Qingdao Hope bio-technology Co. Ltd. (Qingdao, China) Phosphate buffer saline (PBS) was obtained from Beyotime biotechnology Co. Ltd (Shanghai, China). All reagents were used without further purification.

The chemical elemental composition (wt. %) of the AISI 1045 medium carbon steel (Baowu Steel Co. Ltd.) was: 0.45-0.5% C, 0.17-0.37% Si, 0.50-0.80% Mn, ≤0.035% S, ≤0.035% P, ≤0.25% Cr, ≤0.25% Ni and Fe. The AISI 1045 specimens were cut into 10 mm × 10 mm × 2 mm dimension pieces and were gradually ground using a series of silicon carbide abrasive papers. All the specimens were ultrasonically cleaned with deionized water followed by ethanol, for 20 min. Each specimen used in the electrochemical test was welded with copper wire on the unground side, and then the joint was embedded into epoxy resin to ensure that the area of electrochemical measurement was maintained at 1 cm^2. Each surface was degreased with acetone, rinsed with deionized water and dried using a drier, then all specimens were sterilized with UV light for 30 min.

The model MWF was an aqueous O/W emulsion, which was produced by Hinar metal working chemical Co. Ltd. (Changzhou, China) based on the requirements of the industrial standard (GB/T 6144-2010). Biocides were excluded from the formula of the model MWF to make microbial colonization easier. The species of raw materials were limited in order to create a simple emulsion system. The components of the model MWF and their functions are summarized in Table 1.

Table 1. The components of model MWF and their functions

Additive	Component	Molecular formula	Function
Base oil	Mineral oil	 petroleum-based naphthenic oil	Reducing the friction between the workpiece and the tool
Surfactant	Nonionic surfactant	petroleum-based	Suspending oil droplets in water to form an O/W emulsion

Oily agent	Polyethylene glycol ester		Providing a slippery boundary lubricant film
Corrosion inhibitor	Monoethanolamine benzoate		Preventing the corrosion of metal
Buffer agent	Monoethanolamine; Triethanolamine		Maintaining the alkaline pH of the diluted fluid

2.2 Bacterial isolation and identification

The sterilized AISI 1045 steel was immersed in the WMWF for three days, which had been collected from the Shanghai Spaceflight Precision Machinery Institute (Shanghai, China). Dominant bacteria were collected from the surface of the AISI 1045 steel by scraping and transferred into the LB broth culture medium and cultured at 37 ℃ for 48 h. Single dominant colonies were collected and purified by successive streaking on LB broth plates (15 g/L agar and 25 g/L LB broth) [21]. The optical density (OD) absorbance value (λ = 600 nm) of one single dominant colony was determined using an ultraviolet spectrophotometer (TU-1901, Persee, Beijing, China). The OD absorbance of the microbial culture medium exhibited a linear relationship with biomass [22]. The E.Z.N.A. ® soil DNA Kit (Omega Bio-tek, Norcross, USA) was used to extract DNA from microorganisms in the isolated sample in the logarithmic phase. A NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA) was used to measure the final DNA concentration and DNA quality was checked using 1% agarose gel electrophoresis. The bacterial 16S rRNA gene was amplified with 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers, using a thermocycler PCR system (GeneAmp 9700, USA). The PCR products were extracted from a 2 % agarose gel and further purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) and then quantified using Quantifluror ^{TM} – ST (Promega, USA). The purified amplicons were pooled in equimolar concentrations and paired-end sequencing (2 × 300) was performed using an Illumina MiSeq platform (Illumina, USA). The gene sequence was analyzed via the Silva (SSU123) 16S rRNA database. One of the dominant bacteria were identified as *Pseudomonas* sp., strain *P. xiamenensis*, which were used as the bacteria for all following microbial experiments.

2.3 Biodegradation of MWF influenced by Pseudomonas xiamenensis

The model MWF was sterilized in an autoclave at 121 ℃ for 20 min. The 5 % (v/v) 2216E culture medium containing 10^4 CFU/mL of *P. xiamenensis* according to the OD absorbance value, was added to the sterilized MWF. The bacteria-contaminated MWF and controlled MWF were cultured in the incubator at 37 ℃ for 15 days. Analysis of the MWF was carried out at specific time intervals (day 1, 3,

5, 10 and 15). The bioburden of MWF was assessed daily for the first 7 days was established by the flat colony counting method (15 g/L agar and 25 g/L 2216E culture medium) on 2216E culture medium. 2216E culture medium consisted of: 5.0 g/L peptone, 1.0 g/L yeast extract, 0.1 g/L ferric citrate, 19.45 g/L NaCl, 5.98 g/L $MgCl_2$, 3.24 g/L Na_2SO_4, 1.8 g/L $CaCl_2$, 0.55 g/L KCl, 0.16 g/L Na_2CO_3, 0.08 g/L KBr, 0.034 g/L $SrCl_2$, 0.08 g/L $SrBr_2$, 0.022 g/L H_3BO_3, 0.004 g/L Na_2SiO_3, 0.0024 g/L NaF, 0.0016 g/L NH_4NO_3 and 0.008 g/L NaH_2PO_4. A pH meter (FE20, Mettler-Toledo, China) and portable turbidity meter (2100Q, Hach, USA) were applied to measure the pH and turbidity of MWF on the 1^{st}, 3^{rd}, 5^{th}, 10^{th} and 15^{th} days. The total organic carbon (TOC) and total nitrogen (TN) levels were determined using a TOC/TNb analyzer (multi N/C 3100, Analytic Jena AG, Germany).

In addition, specimens of AISI 1045 and electrochemical specimens were immersed in the microbially contaminated MWF, which were then taken out for analysis of corrosion at 15 days. The electrochemical specimens and 80 mL of MWF containing *P. xiamenensis* were transferred to an electrochemical test cell for analysis.

2.4 Corrosion of AISI 1045 in biodegraded MWF containing Pseudomonas xiamenensis

Surface morphological analysis was performed on specimens which had been immersed in MWF, in the presence and absence of bacteria. The AISI 1045 coupon was immersed for 15 days in MWF containing bacteria, then flushed with phosphate buffer saline (PBS) three times. The bacterium and biofilm on the surface of AISI 1045 were immobilized in a 2.5% glutaraldehyde solution for two hours, then the coupon was respectively dehydrated in a series of ethanol dilutions (10%, 30%, 50%, 70%, 90% and 100%) for 20 min. Surface analysis of the specimen involved characterization using a scanning electron microscope (JEOL JSM-7500F, Japan) connected with an energy-dispersive X-ray spectrometer (EDAX). An optical profilometer (Bruker, Contour GT, Germany) was applied to view the surface profile of the carbon steel. Furthermore, another piece of the coupon was stained with 0.001 mg/mL acridine orange (AO) to observe the active bacterium present on the surface of AISI 1045 by fluorescence microscopy (Ti-E, NIKON, Japan). Moreover, the coupon was immersed in concentrated hydrochloric acid for 3-5 s to remove corrosion products (CP) and then immediately immersed in a saturated sodium bicarbonate solution to neutralize the residual acid present on the steel surface. The morphology of the specimen from which the CP had been removed, was obtained by SEM and optical profilometry.

2.5 Electrochemical measurements

The electrochemical behavior of AISI 1045 carbon steel in the microbially contaminated MWF was measured using a three-electrode workstation (CHI-660D, Chenhua, Shanghai, China). In electrochemical tests, the counter electrode (CE), reference electrode (RE) and working electrode (WE) were platinum plate, saturated calomel electrode (SCE) and AISI 1045 carbon steel, respectively. Prior to measurements, the three electrodes were immersed in MWF for a minimum of 1 h, to ensure that a stable open circuit potential (OCP) could be obtained. Electrochemical impedance spectroscopy (EIS)

was carried out at the OCP, with an amplitude of 10 mV and varying frequency between 0.01 Hz and 10^4 Hz. Following electrochemical measurements for 15 days in MWF in the presence and absence of *P. xiamenensis*, polarization curves were tested at a scan rate of 5 mV/s based on the stable OCP.

3. RESULTS AND DISCUSSION

3.1 Bacterial identification

The OD (λ = 600 nm) absorbance value of the isolated strain cultured in the 2216E culture medium, is shown in Fig. 1a. Results show that the concentration of bacteria dramatically increased from 20 h to 108 h and decreased after 120 h. 16S rDNA analysis of bacteria cultured for 100 h, indicated that the isolated dominant strain was *P. xiamenensis*, with the dsr-amplified specific gene presenting 100% similarity. *P. xiamenensis* is a Gram-negative, non-pigmented, rod-shaped bacterium, with the wild type collected from activated sludge in China [23]. *Pseudomonas sp.* has been shown to have chemical and biochemical activity in different chemotaxonomic studies, producing compounds such as quinones, fatty acids, proteins and polar lipids [24–27].

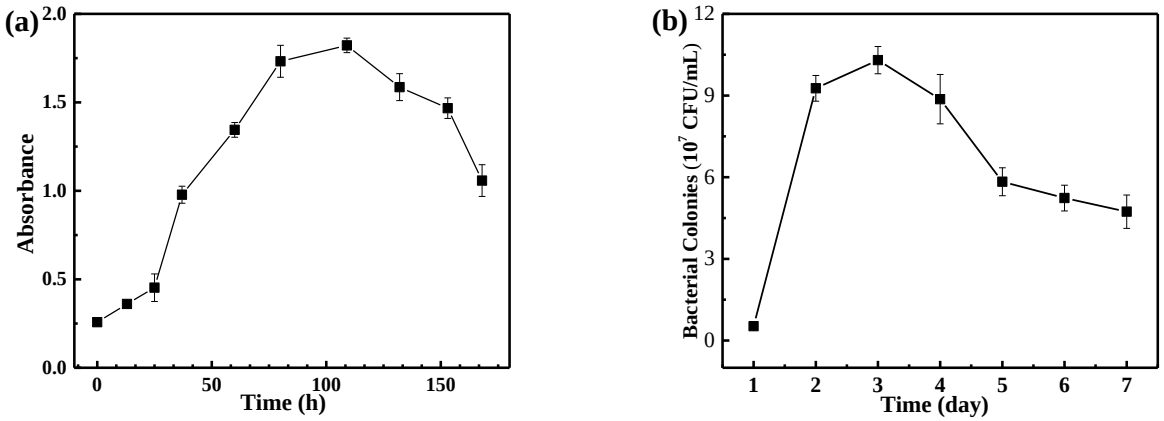


Figure 1. Enrichment of *P. xiamenensis* in different culture media (a) OD (λ = 600 nm) absorbance of *P. xiamenensis* in 2216E culture medium during 7 days of enrichment at 37 ℃. (b) Microbial colonies of *P. xiamenensis* in MWF during 7 days of enrichment (initial concentration of 10^2 CFU/mL *P. xiamenensis*).

3.2 Biodegradation of MWF influenced by P. xiamenensis

Fig. 1b shows the bacterial growth of MWF containing an initial concentration of 5 % (v/v) 10^4 CFU/mL *P. xiamenensis* (i.e., an initial bioburden of 10^2 CFU/mL) during the initial 7 days. Counts of bacteria were approximately 10^6 CFU/mL on the first day, increasing to 9.2×10^7 CFU/mL on the second day and reaching a maximum of 10^8 CFU/mL on the third day. After four days, the bacterial concentration decreased marginally to 4×10^7 CFU/mL.

Fig. 2 presents the temporal variation in characteristics (pH, turbidity, TOC and TN) of MWF containing *P. xiamenensis*. The pH in fresh MWF was 9.88, decreasing to 8.3 from day 1 to day 3, gradually increasing slightly after day 5 and then remaining around 8.6 from day 10 to 15 (Fig. 2a). A growing tendency of turbidity was observed, with an initial turbidity of 365 NTU, increasing to 616 NTU after 15 days (Fig. 2a). Both TOC and TN exhibited a declining tendency, from 34.5 mg/L and 1036 mg/L for fresh MWF, to 26.8 mg/L and 723.6 mg/L after 15 days of biodegradation, respectively (Fig. 2b). Solution pH is an important parameter in the MWF in related to its anti-corrosion capability. Carbon steel presents a passive state in an alkaline solution which can prevent the metal from being corroded [28–30]. Therefore, usually MEA or TEA is added to MWFs to maintain a solution pH between 8.0 to 9.5 [31]. Previous research has proven the biodegradation of MEA or TEA under the influence of *P. xiamenensis.* [15, 32]. *P. xiamenensis* may utilize TEA as a nitrogen sources in this kind of emulsion system, oxidizing TEA to nitrogenous inorganic compounds or nitrogenous gas [33].

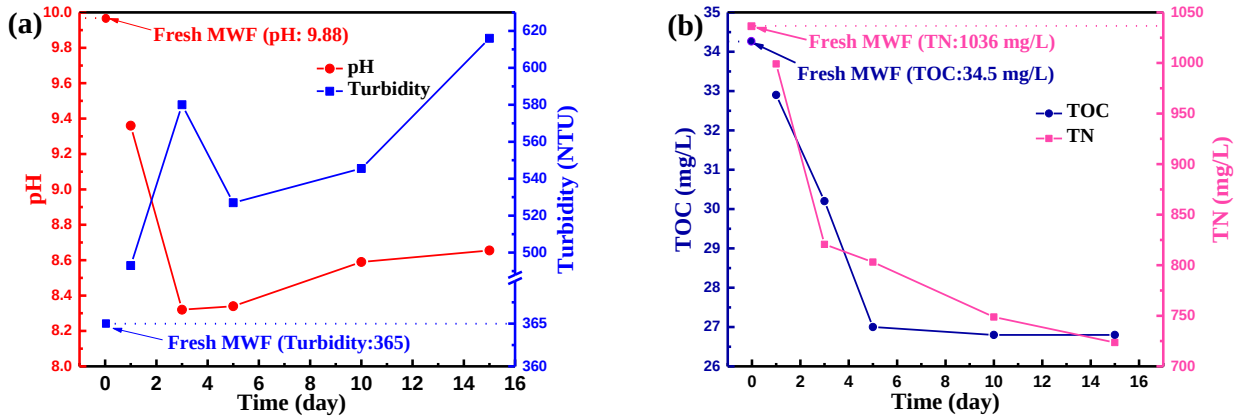


Figure 2. Temporal trend in biodegradation of MWF containing *P. xiamenensis* with varying (a) pH and turbidity; and (b) TOC and TN (initially 10^2 CFU/mL of *P. xiamenensis* in MWF).

Petroleum-based naphthenic oil is a long-chain hydrocarbon component of MWF, as shown in Table. 1. It is difficult to degrade naphthenic oil due to its stable chemical structure as compared with other carbon nitride organic additives. Nitrogenous compounds were preferentially degraded, as shown by the TN degradation rate in Fig. 2b. In the MWF formula, all nitrogen sources were provided by the nonionic surfactant and buffer agents (TEA, MEA). Rabenstein et al. also reported that the surfactant and buffer agent was easier to be degraded than mineral oil [33]. Stable O/W emulsions in the MWF are important in the machining process, maintaining the cutting tool in a continuous processing condition. The MWF amphiphilic surfactant substances (emulsifiers) form stable O/W emulsions, by decreasing the surface tension of naphthenic oil when MWF was diluted by water and maintaining the stabilization of dispersed O/W emulsion systems. [34]. The stability of emulsions affects droplet size distribution [35], with the turbidity accordingly related to the droplet size of emulsions [36, 37]. The biological, thermal and chemical degradation of emulsifiers could result in the coalescence of droplets, leading to increased turbidity of MWF [38]. The rate of decline in pH was much lower than the rate of increase in turbidity, indicating that emulsifiers can be preferentially decomposed by *P. xiamenensis* (Fig. 2a). MacElwee et al. [14] stated that bacteria from the genus *Pseudomonas sp.* had the ability to secrete EPS

for demulsification. Although microorganisms may contribute to turbidity, the decrease in biomass after day 5 (Fig. 1b) and the increase of turbidity (Fig. 2a), indicated that the increase in turbidity could be mainly attributed to the biodegradation and demulsification of *P. xiamenensis* after day 5.

3.3 Characterization of surface morphology

Fig. 3 shows the SEM and EDS imaging results of the AISI 1045 coupons after being immersed in MWF containing *P. xiamenensis* for 15 days. As shown in Fig. 3a, rod-shaped bacteria adhered onto the carbon steel surface. Results also show that a thick non-homogeneous biofilm had been formed on the steel surface by day 15. The width of cracks were approximately 4 μm, and the dimension of agglomerated films was approximately 30 × 30 μm. EDS results show that the proportion (%) of C, O, Si, P, Ca and Fe, were 29.68%, 49.48%, 1.79%, 1.65%, 1.42% and 15.95%, respectively (Fig. 3b). The fluorescence microscopy imaging of AISI 1045 carbon steel after 15 days of immersion in MWF containing *P. xiamenensis*, is shown in Fig. 3c. The stained bacterial DNA exhibits green fluorescence under fluorescence microscopy, showing that bacteria maintained high biological activities in the biofilm. There were numerous bacteria present in the steel surface film, according to the fluorescence intensity.

Fig. 4 shows the optical profile of AISI 1045 carbon steel after being immersed in MWF containing *P. xiamenensis* for 15 days. The maximum surface fluctuation of AISI 1045 specimens was approximately 57 μm where the biofilm and CP had not been removed (Fig. 4a and 4b). The cover layer on the steel surface was not uniform or dense, presenting numerous cracks and imperfections dispersed across the layer, which was in agreement with the SEM results. A severe pitting corrosion phenomenon was observed on the steel when the CP and biofilm were removed (Fig. 4c, 4d). There were some CP residues and products of acid-base neutralization reactions present on the surface of the specimen. But matrix scratches were clearly visible, as shown in the figure. The maximum surface fluctuation was approximately 15 μm except for the influence of residues. Numerous pits presented on the surface of the steel, and the average pit size was approximately 20 μm diameter and 15 μm depth. The worse pitting corrosion occurred in the presence of pit joints, where the hole size diameter of pit joints increased 4- to 5-fold compared to the average pit size.

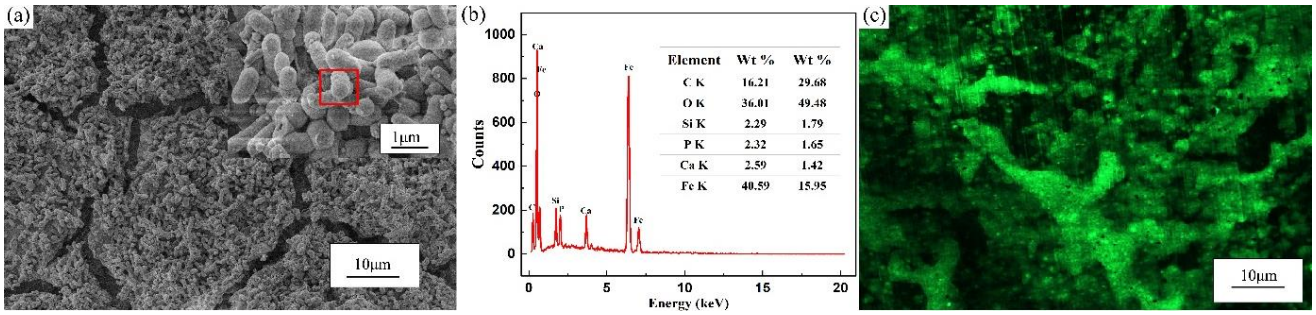


Figure 3. Morphologies of biofilm on AISI 1045 after being immersed in MWF with *P. xiamenensis* for 15 days. (a) SEM images at low resolution, with insert showing high resolution; (b) EDS characterization of biofilm; (c) Fluorescence micrograph of biofilm after AO staining

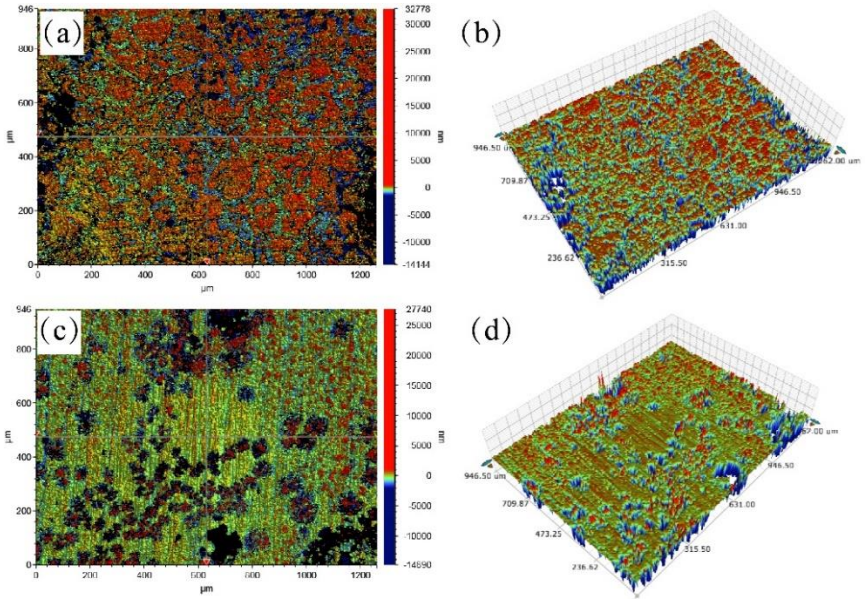


Figure 4. Optical profiler images of AISI 1045 immersed in MWF containing *P. xiamenensis* for 15 days before the biofilm was removed (a, b) and after the biofilm removed (c, d).

3.4 Electrochemical measurements

Fig. 5 shows the polarization curves of AISI 1045 in microbially contaminated MWF after 15 days of immersion with and without *P. xiamenensis*. The electrochemical fitting parameters are shown in Table 2. Corrosion potential (E_{corr}) of the steel in biodegraded MWF was more negative compared with the corrosion potential of MWF without adding *P. xiamenensis*. The corrosion current density ($\boldsymbol{i}_{corr}$) of the AISI 1045 carbon steel in MWF with *P. xiamenensis* (0.157 $\mu A \cdot cm^{-2}$) was almost 3-fold higher than in the control MWF (0.052 $\mu A \cdot cm^{-2}$). The polarization resistance (R_p) of steel immersed in the microbially contaminated MWF was 303.1 $k\Omega \cdot cm^2$ while the steel in MWF without bacteria was 709.7 $k\Omega \cdot cm^2$. The significant shifting of E_{corr} in microbially contaminated MWF can be explained by the formation of the CP and biofilm layer [39, 40]. The presence of a stage in the anodic process implies *P. xiamenensis* influenced the anodic reaction, probably because the biofilm restricted the transfer of iron to ion form. Interestingly, the anomalous electrochemical behavior of i_{corr} and R_p indicated that the steel was more aggressive when being exposed to MWF containing *P. xiamenensis*. The MWF presented different characteristics when enriched with *P. xiamenensis* for 15 days. The emulsion with microorganisms was characterized as forming larger droplet sizes, contributing to the biodegradation of surfactants, which changed the stability of emulsions and consequently resulted in the formation of an oil phase on the top of the solution. Furthermore, when the coupon was added to the solution, it changed from an O/W emulsion into an aqueous solution, leading to a higher possibility of corrosion for AISI 1045 carbon steel.

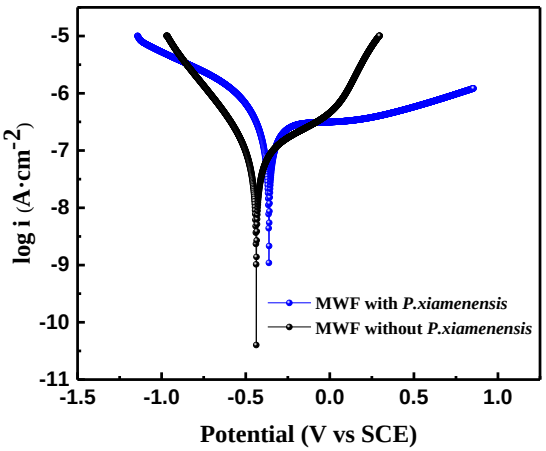


Figure 5. Polarization plot of AISI 1045 in the MWF in the presence and absence of *P. xiamenensis*

Table 2. The electrochemical parameters of polarization curves

Media	E_{corr} (V vs SCE)	I_{corr} ($\mu A \cdot cm^{-2}$)	R_p ($k\Omega \cdot cm^2$)	β_c (V/dec)	β_a (V/dec)
MWF with P.x	-0.373	0.157	303.1	-0.167	0.318
MWF without P.x	-0.316	0.052	790.7	-0.247	0.155

The EIS results shown in Fig. 6 include the typical Nyquist plots and Bode plots. The electrochemical impedance of AISI 1045 carbon steel in fresh MWF exhibited lower corrosion resistance than in other media. The diameter of capacitive semicircles on day 15 without *P. xiamenensis,* increased significantly compared to the fresh MWF. Furthermore, a semi-finite Warburg element (W) appeared on day 15 with exposure to *P. xiamenensis*, which illustrated that electron transfer was controlled by diffusion. The appearance of the Warburg element (W) demonstrated the existence of a non-planar surface and non-uniform adsorbed layer. The time constants of AISI 1045 carbon steel in the three different media types are shown in Fig. 6b and the electrochemical fitting parameters are shown in Table 3. The solution impedance (R_s) of MWFs presented different values under the influence of *P. xiamenensis*, with fresh MWF exhibiting the biggest R_s value relative to other MWFs. R_{ct} is a criterion which presents the resistance of electron transfer between the film and the matrix metal. Results show that hindrance of electron transfer of steel after 15 day exposures to MWF with *P. xiamenensis* was more prominent than with exposures to fresh MWF or 15 day exposures to MWF without *P. xiamenensis*.

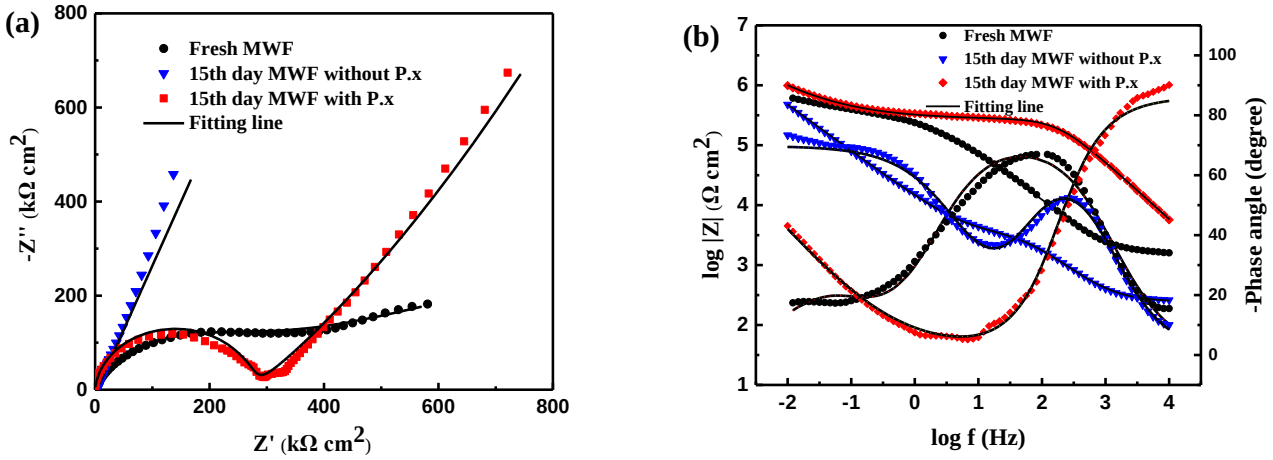


Figure 6. Nyquist and Bode plots of AISI 1045 in three different immersion media types, fresh MWF and MWF in the presence or absence of *P. xiamenensis*

Table 3. The impedance parameters of AISI 1045 in three different immersion media types, fresh MWF and MWF in the presence or absence of P. xiamenensis.

Media	R_s ($\Omega \cdot cm^2$)	Q_f ($\times 10^{-5} F \cdot cm^{-2}$)	n_f	R_f ($k\Omega \cdot cm^2$)	Q_{ct} ($\times 10^{-5} F \cdot cm^{-2}$)	n	R_{ct} ($k\Omega \cdot cm^2$)	W ($s^{1/2}/m\Omega \cdot cm^2$)
Fresh MWF	185.35	4.57	0.82	29	0.849	0.71	158	-
Day 15 without *P. xiamenensis*	136.81	0.236	0.87	51	1.80	0.76	188	-
Day 15 with *P. xiamenensis*	81.13	7.93	0.93	215	2.74	0.95	213	6.2×10^{-3}

The electrochemical impedance was analyzed in two circuit models according to the chi-square value ($\chi^2 < 2.2 \times 10^{-3}$), which was satisfied by the simulation quality. As shown in Fig. 7, The circuit of metal on day 15 in fresh MWF and MWF without *P. xiamenensis,* was R(QR)(QR). The additives film developed on the steel surface after being immersed in fresh MWF and following 15 days of MWF without *P. xiamenensis*, was due to the MWF containing the monoethanolamine benzoate and nonionic surfactant as the corrosion inhibitor and surfactant. Monoethanolamine benzoate is a kind of film-forming inhibitor which affects polar groups, such as -OH [19, 41]. Inhibitors reduce the anodic and cathodic reaction, by forming a thin protective film on the surface of AISI 1045. The surfactant can adsorb on the steel surface under the effects of chemisorption and physisorption [42–44]. With adsorption of the inhibitor and the surfactant, a mixture of additives formed films covering the steel surface in fresh MWF and following 15 days of exposure to MWF without *P. xiamenensis*. The corrosion rate was decreased by the extension of immersion which was contributed to by the thickening of the additive film. The R_f values of steel in fresh MWF and following 15 days of exposure to MWF without *P. xiamenensis* were 21 and 59, respectively, proving that the anticorrosion effect was reinforced by increasing immersion time. In contrast, the biodegraded MWF was enriched with numerous bacteria,

which led to the consumption of surfactants and corrosion inhibition. The presence of W in the equivalent electrical circuit indicates that the corrosion mechanism of the steel had changed. The R_f value following 15 day exposures to MWF with *P. xiamenensis* was 5-fold greater than the controlled MWF, implying a biofilm had formed on the steel surface. EPS which was produced by *P. xiamenensis* can utilize Ca^{2+} in the MWF to form an organic-inorganic film on the substrate [45–47]. According to the EDS analyses of AISI 1045 in Fig. 4b, the observance of Ca, C and O confirm that biomineralization occurred on the steel surface. However, the utilization of Ca^{2+} was too low to generate a compact film so that the morphology of biofilms in Fig. 3 and Fig. 4 exhibited the cracked film on the steel matrix. The localized corrosion markedly accelerated the pitting of AISI 1045 carbon steel. High levels of carboxylic acids exacerbated the size of pitting of the steel, even though the steel showed higher resistantance to corrosion in terms of electrochemical behavior.

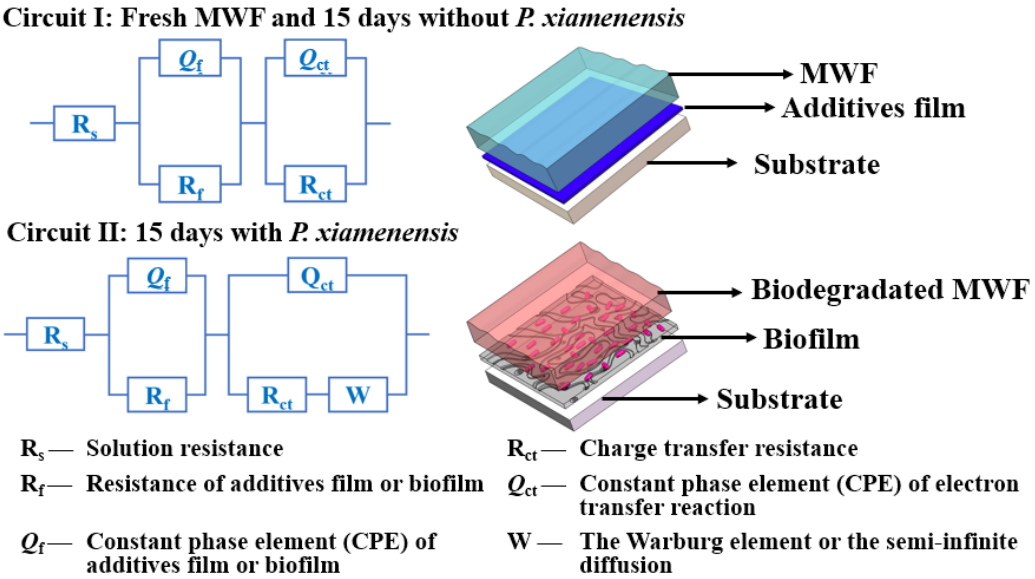


Figure 7. Schematic diagram and equivalent electrical circuits used for fitting of EIS diagrams

4. CONCLUSIONS

The dominant bacteria *P. xiamenensis* was successfully isolated from an industrial WMWF. The measurements of pH, turbidity, TOC and TN of microbially contaminated MWF, indicated a preferential degradation of nitrogenous emulsifiers and buffer agents in the MWF. The additives film was replaced by a biofilm on the steel surface and bacteria utilized organic substances and Ca^{2+} to increase the thickness of the biofilm. The substrate was vulnerable to high EPS concentrations produced by microbial metabolism. After 14 days of immersion, a high level of pitting occurred on the surface of AISI 1045 carbon steel and the corrosion rate of steel in the MWF containing *P. xiamenensis* was approximately 3-fold greater than in the controlled MWF.

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