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IMPACT AND CORROSION BEHAVIOR OF MILD STEEL IN THE PRESENCE OF *PENICILLIUM CHRYSOGENUM*

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ABSTRACT: *The growth of fungi on the surface of metals has great influence on their structural integrity and failure. The possibility of some fungal species to grow on metal surfaces is determined by their secreted metabolites which aid their adaptation to new environmental and nourishment conditions. The aim of this study is to determine the influence of Penicillium chrysogenum on the morphological and corrosion behavior of mild steel using gravimetric and potentiodynamic techniques. Mild steel coupons were contaminated with the above-mentioned fungus in Petri dishes with nutrient medium imitating organic pollution. The gravimetric and electrochemical analysis results revealed that the corrosion rate (CR) and weight loss (ΔW) of the mild steel increased with time. The cumulative corrosion rate (ΣCR) increased from 1.74mpy in the absence of P. chrysogenum to 7.58mpy in the presence of the fungus while the corrosion current density (I_{corr}) increased to 258.6μAcm^2 from 187.9 μAcm^2.*

KEY WORDS: Biocorrosion, fungi, structural integrity, metal coupons, corrosion rate

INTRODCTION

Fungi play an important biogeochemical role in the biosphere and are intimately involved in the cycling of elements and transformation of both organic and inorganic substrates (Lugauskas *et al.* 2009). They are ubiquitous members of the sub-aerial and subsoil environments, and often become a dominant group in metal rich or metal polluted habitats. Fungi have ability for growth under extreme environmental conditions which allows their successful colonization of metal surfaces. The overgrowth of metallic surfaces with fungus mycelium is closely related to electrochemical processes (Juzeliunas *et al.* 2007).

Microorganisms including fungi are able to depolarize both the cathodic and anodic sites of a metal either directly by their metabolic activities or indirectly by excretion of chemically reactive products (Miller, 1981; Widdel 1992). They are particularly corrosive as they grow in colonies or films attached to the metal surface with their mycelia and thereby create local electrochemical cells with highly stimulated reactions. As a result, corrosion by microorganisms often occurs as pitting, which is usually more severe than corrosion processes that are evenly distributed over the metal surface (Hamilton and Lee 1995; Crod-Ruwisch, 2000; Lee *et al.* 1995). Also, when fungi

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grow on metal surfaces, they not only can consume the nitrate and sulfur accumulated on the eroded poles (such as iron) but also the hydrogen, oxygen and other gaseous products formed on the metals hence depolarizing the metals and enhancing corrosion (Obuekwe, 1981). Therefore, microbial corrosion processes at metal surfaces are associated with microorganisms or the products of their metabolic activities (Beech et al 1999). These microbial metabolic products can affect cathodic and or anodic reactions thereby altering the electrochemistry at the metal/biofilm interface (Hector and Liz 2005; Imo *et al.* 2016).

Studies of fungi overgrowth on metal surfaces in open natural environments shows that fungi are most intensively evolve in places where the metal comes in contact with water and air. It has been stated that the electrical characteristics of steel, aluminum and titanium can be worsened by the growth of some species of fungi belonging to the genera *Apergillus, Penicillium, Sporotrichum, Cladosporium, Paecilomyces* (Stokes and Lindsay 1997). On land and aqueous environments, metals are corroded not only by purely chemical or electrochemical reactions but also by metabolic activities of microorganisms (Koch *et al* 2002; Uhlig, 1985) of which fungi play a very great role.

Penicillium chrysogenum formerly known as *Penicillium notatum* is a species of fungi in the family of Trichocomaceae. It can be found in damp or water-damaged buildings (Anderson *et al.* 2011). The mycelium of *P. chrysogenum* typically consists of a highly branched network of multinucleated septate, usually colorless hyphae. Many branched conidiophores sprout on the mycelia bearing individually constricted conidiospres commonly known as molds, which are among the main causes of spoilage (Samson *et al* 2004). *P. chrysogenum* usually reproduces by forming dry chains of spores (or conidia) from branch –shaped conidiospores. Their conidia are blue-green and the mold sometimes extrudes a yellow pigment. However, *P. chrysogenum* cannot be identified based on colour alone. A careful observation of morphology and microscopic features are needed to confirm its identity. Salo, (2016) observed that the production of metal ion binding siderophores is typical among *P. chrysogenum* that grows naturally at various challenging environments. This study focuses on the influence of *P.chrysogenum* on the corrosion behavior of mils steel. We also analyzed the corrosion results using gravimetric and potentiodynamic polarization methods to ascertain the influence of the fungus on the anodic and cathodic partial reactions of the corrosion processes.

MATERIALS AND METHODS

Our experimental approach involves isolating *P. chrysogenum* from corroding pipeline, followed by inoculating it on mild steel coupons in Petri dishes with nutrient agar imitating organic pollutant (Lugauskas *et al.* 2009). Next was the assessment of the corrosion behavior of the metal using gravimetric and electrochemical methods.

Metal preparations

The metals for the gravimetric studies were cut into coupons of specific dimensions 2 x 2x 0.14cm. The metal coupons were wet polished with silicon carbide abrasive paper

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(from grade No 400-1000), to produce a smooth metal surface and finally polished to mirror finish surface. The refined coupons were rinsed with distilled water, dried in acetone and warm air, weighed and stored in moisture free desiccators prior to use (Oguzie *et al.*,2012; 2013).

Preparation and standardization of inoculum: The inoculum used for the work was prepared according to the method outlined by Petrikkou *et al.* (2001). From the stock culture stored in the fridge, isolates were subcultured on potatoes dextrose agar PDA and incubated at room temperature (28±2ºC) for 7 days on the lab bench. Thereafter, the surface of the agar was flooded with 50 mL sterile distilled water and the sporulated aerial mycelia were scraped with a loop. The suspensions obtained were then filtered to remove the hyphae and thereafter the filtrate which contained the spores were collected in sterile tube. The spore suspensions were adjusted to spore concentration of 10^6 spores/mL by microscopic enumeration with a cell counting hematocytometer (Neubauer Chember; Merck, S.A., Madrid, Spain).

Determination of the influence of *P. chrysogenum* on the corrosion of mild steel

The contact of the metals with the fungi was investigated. The prepared metal coupons were placed in Petri dishes containing 20 mL malt extract agar MEA medium and supplemented with streptomycin (5µg/mL). Thereafter, 0.4 mL standardized suspensions of the respective fungal isolate were spread over the plates and metal coupons. In the control (K), metal was placed on MEA medium but not inoculated with fungi. The plates were incubated at room temperature (28±2ºC). The entire experiments were uniformly prepared in triplicates, labeled accordingly and inserted on the same day for. At 10 days interval, the plates were analyzed for the following: (*i.*) Macroscopic and microscopic examination of the metal coupons. (*ii*.) Gravimetric corrosion measurement.

Macroscopic and Microscopic examination of the metals

Each metal coupon retrieved at 10 days interval was carefully examined to determine the intensity of fungal growth and contact with the metals in accordance with the method outlined by (Lugauskas *et al.* 2009).

The intensity of fungal growth and metal deterioration was assessed by physical observation of the coupons. The metal coupons were then observed on the light microscope to ascertain the growth of the fungi on the surface of the coupons. Morphological changes on the metals were evaluated using an optical microscope with a CCD camera at about 50X magnification and the magnitude of marker was 10µm per 1 cm of the photograph.

Gravimetric corrosion measurement

i. Weight loss measurement

Weight loss of the metal coupons with respect to time was conducted using the method outlined by Oguzie *et al.* (2013). The coupons were retrieved at 10days intervals

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progressively for 60days. Thereafter the metal coupons were scraped with spatula, washed with distilled water, dried on the lab bench and weighed. The weight loss was taken to be the difference between the weight of the coupons at a given time and its initial weight. Average values for each experiment were obtained and used in subsequent calculations.

Calculations:

Weight loss (ΔW) = W_i - W_f

Where W_i = Initial weight; W_f = Final weight

ii. **Determination of corrosion rate**

Corrosion rate (CR) is used to determine the rate at which a metal will corrode over a period of time.

$$\mathbf{CR} = \frac{\mathbf{k\Delta W}}{\mathbf{A\rho t}}$$

A= Exposed surface area =2(LW+LH+HW) cm^2

Where L= length of the coupon; W= width of the coupon; H= height of the coupon or thickness k = corrosion rate constant (143,700mpy); ρ =density of metal coupon (g/cm^3); ΔW= weight loss of coupon (g); t = time (days)

Electrochemical experiment

Potentiodynamic polarization experiments were conducted to ascertain first, the influence of the fungus on the kinetic of the anodic and cathodic partial reactions of the corrosion processes and secondly the effects of the fungi on the electrochemical properties of the metal and the resulting corrosion behavior. The test was carried out in a standard three-electrode glass cell of 500 ml capacity using Electrochemical System workstation (PAR 263). A graphite rod served as counter electrode and, a saturated calomel electrode (SCE) was used as reference electrodes. A mild steel and aluminum specimen of 1 cm^2 dimension were used as working electrode. Electrochemical measurements were carried out at 30±1ºC, using standard procedures as outlined by Oguzie *et al.* (2013), in aerated solutions at the end of 1800s of immersion, which allowed the open circuit potential (OCP) values to attain steady state. The polarization (PDP) experiments were then conducted at a scan rate of 0.333mV/s. The potential range employed was -250mV to + 300mV versus corrosion potential. Powersuite software was used in analyzing the polarization data.

RESULTS

The microscopic examination of the metal coupons after 10 days exposure to fungus revealed that fungal conidia and colonies were sparsely scattered on the mild steel surfaces. However, after 60 days, the macroscopic examination showed that the fungus grew on the edges of the metal covering most part of the coupons. The picture shown

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in Figures 3 (MS-PC) shows corrosion spots on the surfaces of mild steel coupons exposed to *P. chrysogenum* compared to the control (MS). Visible colour changes were observed on the surfaces of the mild steel influenced by *P. chrysogenum*.

The gravimetric results of the influence of *P. chrysogenum* on the corrosion behavior of mild steel are shown in Table 1. The data presented are average of triplicate determinations. The results show that the growth and attachment of *P. chrysogenum* significantly influenced the metal corrosion progressively over the period. Figures1 and 2 shows the variations in ΔW and steady increase in CR with exposure time. The plots show that *P. chrysogenum* effectively increased the corrosion rate of mild steel.

Table 1: The influence of *P.chrysogenum* on the corrosion behavior of mild steel

Fungi	Metal density (gcm^{-3})	Initial weight(g)	Final weight(g)	Δ weight (g)	Surface area (cm^2)	Exposed time (Days)	Corrosion rate CR (mpy)
P.chrysogenum	7.9	8.583	8.582	0.001	9.12	10	0.19
	7.9	8.577	8.572	0.005	9.12	20	0.49
	7.9	8.866	8.842	0.020	9.12	30	1.30
	7.9	8.822	8.792	0.030	9.12	40	1.50
	7.9	8.517	8.467	0.045	9.12	50	1.80
	7.9	8.475	8.405	0.070	9.12	60	2.30

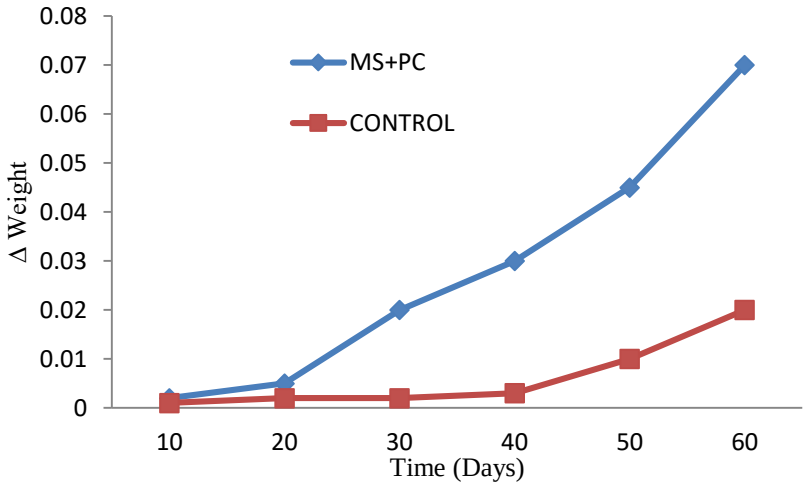

Figure 1: Weight loss of mild steel in the presence of *P.chrysogenum*.

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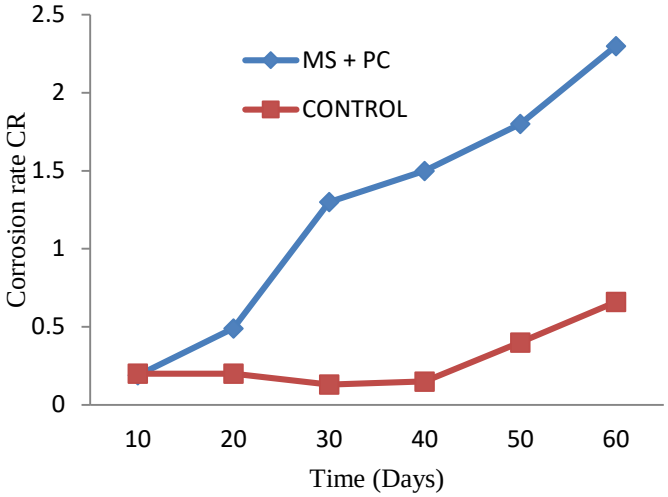

Figure 2: Corrosion rate of mild steel in the presence of *P. chrysogenum*.

The result of the intensity of fungal growth, metal oxidation and surface changes after 60 days exposure to influence of *P. chrysogenum* showed significant prove of metal oxidation and surface changes with dark spots like pits (Figure 3).

MS-PC MS

Figure 3: Microscopic view of changes in mild steel after 60days exposure to *P.chrystogenum*

Legends: MS-PC= Mild steel exposed to *P.chrysogenum* MS= Control

Electrochemical measurement

The results of the potentiodynamic polarization test and the corresponding polarization data is shown in Table 2. From the data, the I_{corr} is proportional to CR. Figure 4 shows the potentiodynamic polarization curve of mild steel corrosion influenced by *P. chrysogenum*. The I_{corr} in the presence of *P. chrysogenum* increased to 258.6µA/cm^2 relative to the control (187.9µA/cm^2) in the absence of the fungus after 60days incubation. The i_a was also higher (107µA/cm^2) in the presence of *P. chrysogenum* compared to the i_a (105.3 µA/cm^2) in the absence of the fungus.

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Table 2: Polarization data for mild steel in the presence of *P.chrysogenum*.

Fungi	**I_{corr} ($\mu A/cm^2$)**	**E_{corr} mV Vs SEC**	**b_a**	**b_c**
Penicillium chrysogenum MS3	258.6	−490.2	107.0	69.5
Control MS4	187.9	−491.8	105.3	69.2

Legends: MS3 = Mild Steel *Penicillium chrysogenum;* MS4 = CONTRO

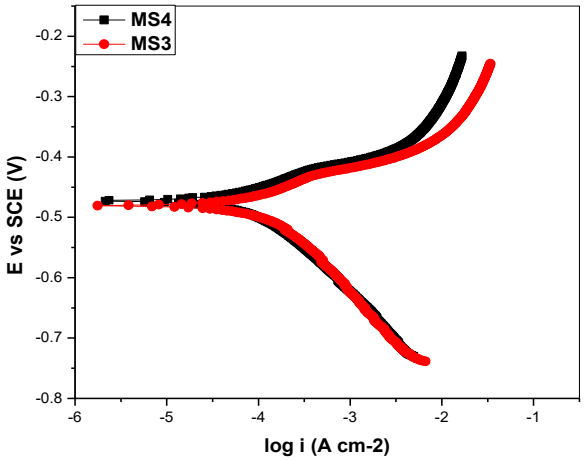

Figure 4: Potentiodynamic polarization curves of mild steel in the presence of *P. chrysogenum.*

DISCUSSION

P. chrysogenum grew on the surface of mild steel with its mycelia covering most part of the metal. However, the growth was most prominent after 60days of incubation. Lugauskas *et al.* (2009) reported the growth of *Penicillium* species on the surface of metal plate in Petri dishes filled with sterile agar medium of malt extract low in nutritive value. The growth of the fungus on mild steel shows its ability to survive on the surface of the metal. Salo, (2016) observed that *P. chrysogenum* could grow naturally at various challenging environment. Little *et al.* (2001) reported fungal growth on interiors of some wooden spools stored outside from which the isolated *Aspergillus* and *Penicillium* species.

The results of the influence of *P. chrysogenum* on the corrosion behavior of mild steel after 60 days exposure showed that the growth and attachment of this fungus significantly influenced its CR over the period as evident in the increase in CR and ΔW. For example, the CR of mild steel exposed to *P. chrysogenum* increased from 0.19 to 2.3mpy within 60 days of exposure. Similarly, there was also a corresponding increase in ΔW observed for the fungus during the 60 days exposure. Remarkably the CR increased with increase in time. This was very pronounced after 30 days of exposure to the fungus and then continued progressively for the rest of the period. This observation

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is typical of a metal that does not demonstrate passivity effects. Similar observations have been reported (Agarry and Salam 2016). Videla, (2002) reported that corrosivity increased with contact time due to accumulation of metabolites under fungal colonies attached to metal surfaces. Results of the gravimetric analysis showed that *P. chrysogenum* was corrosive with the ∑CR and ΔW of 7.58±0.79mpy and 0.17±0.02mpy respectively.

The results of the gravimetric study in the absence of the fungus were lower compared to the values obtained when the metals were exposed to fungus. These results show that the CR might have been influenced by the growth of fungi on the surface of the metals. The increase in CR may also be as a result of the creation of oxygen concentration or differential aeration cell caused by the patchy growth and distribution of fungal colonies and their metabolites on the metals (Beech *et al* 2004). Juzeliunas *et al.* (2007) reported that the overgrowth of metallic surfaces with fungus mycelia was closely related to electrochemical processes. It is also possible that since corrosion is an electrochemical process, the increase in CR observed could be due to fungal growth on the metals. To further support the results obtained from this study, Stokes *et al* (2002) stated that the electrical characteristics of steel and aluminium can be worsened by growth and attachment of some species of fungi belonging to the genera *Aspergillus* and *Penicillium*. Based on their metabolic activities, fungi can oxidize metals. Imo *et al.* (2018) reported the influence of *Aspergillus fumigatus* on the corrosion behaviour of mild steel and alluminium. Metal oxidizing fungi like *P. chrysogenum* generally produces aggressive compounds that induce corrosion damage on metals. It is possible that the production of chelators also known as siderophores by this fungus may have contributed to the corrosion of the metal.

The potentiodynamic polarization curve (Fig. 4) of mild steel in the presence and absence of *P. chrysogenum* showed no distinct difference on the cathodic branch. The anodic branch changed quite slightly suggesting that the anodic site was more sensitive to corrosion with the resultant metal dissolution. Beech and Sunner (2004) reported that I_{corr} is proportional to corrosion rate. And the higher the anodic current i_a the higher the anodic reaction leading to metal dissolution (Little and Lee 2007). It can also be observed that the I_{corr} increased in the presence of the fungus when compared with the values observed in the absence, indicating an increase in corrosion reaction. This suggests that the presence of the fungus and their metabolites might have induced electrochemical activities on the mild steel. Although the detailed activities of the fungi metabolites were not investigated in this study, it is possible to make some inferences considering known functions of these metabolites on the corrosion of metals. Qing *et al.* (2007) pointed out that metabolic by-products and biofilm formation accelerated pitting and corrosion rate of magnesium alloy in artificial seawater. Mansfield *et al* (2002) used potentiodynamic polarization technique to examine the overall corrosion behavior of a corrosion system. The authors observed that increase in I_{corr} was due to the influence of microorganisms on the rate of the anodic and cathodic reactions. There was also a substantial shift of corrosion potential (E_{corr}) towards noble values that occurred throughout the period of exposure of the mild steel to *P. chrysogenum* (Table

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2). The shift to positive potential observed correlates with the growth of the fungus on the mild steel when compared with –491.8mV/SEC obtained in the absence of the fungus. A similar observation was reported by Faisal *et al.* (2013). The potential shift clearly supports the findings that the activities and growth of fungi species enhanced the redox quality of the medium and accelerated the metal dissolution. The positive shifts in E_{corr} may also be as a result of ennoblement which is an indication of potential corrosion. Ennoblement in microbiologically influenced corrosion has been acknowledged by different investigators as probably the most notable phenomenon in microbial influenced corrosion studies (Faisal *et al* 2013; Little *et al* 2001; Videla, 2003). It has been attributed to the microbial colonization and biofilm formation which collectively result in organometallic catalysis and acidification of the metal surface which promotes pitting corrosion as was observed on the mild steel in the presence of the fungus.

CONCLUSION

Atmospheric corrosion of metals and their alloys is controlled by external factors which include microscopic fungi. Fungi are most often the initial colonizers of metal surfaces. The results of this study shows that *P.chrysogenum* had pronounced effects on the corrosion behavior of mild steel. Microscopic fungi produce various metabolites which destruct the surface of steel. Some of these fungi species are capable of developing under strict environmental conditions that are unfavorable for other species development. The influence of any fungus on metal depends on the peculiarities of individual metal and the ability of the fungus to develop under extreme conditions on the metal surface. The results obtained in this study are consistent with our hypothesis that the growth of fungi could influence the corrosion of mild steel and that the rate of corrosion is proportional to the length of exposure.

Conflict of Interest

The authors declare that there is no conflict of interest.

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