The role of fungi on diesel degradation, and their influence on corrosion of API 5LX steel

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MICROBIAL CONTAMINATION of fuels has been the cause of intermittent operation problems and corrosion throughout the world for many years, and more recently the frequency and severity of the cases appears to have been dramatically increasing. This study emphasizes the role of fungi on diesel degradation and its influence on corrosion of mild steel: fungal strains were isolated from diesel oil collected from storage tanks and were identified as *Penicillium sp.*, *Candida sp.*, and *Aspergillus sp.*. The degraded diesel was characterized and analyzed by Fourier transform infra-red spectroscopy (FTIR) and nuclear magnetic resonance (NMR). The corrosion studies were carried out by gravimetric methods, and the product of the corrosion analysed using XRD to find out the nature of the corrosion product. The biodegradation and corrosion studies showed that all the strains were aliphatic protons and aromatic protons in refined diesel, capable of oxidizing ferrous ion into ferric oxide. This is the first report that discloses the involvement of fungal strains on biodegradation of diesel and its influence on corrosion.

MANY STUDIES have shown the importance of microbial tampering of stored hydrocarbon fuels, which leads to the blocking of pipelines and filters, reduces fuel quality, and results in corrosion of pipelines [1-3]. The stored refined or crude oils are transported by pipeline for both marketing and refining, and petroleum products are stored above ground in steel or lined concrete tanks, and microbial contamination of fuels has been the cause of intermittent operational problems throughout the world for many years. The fungal contamination and decomposition of hydrocarbons are well documented phenomena and studied by several investigators [4-12]. The volume of water required for microbial growth in hydrocarbon fuel is extremely small. For example, *Cladosporium resinae*, the kerosene fungus, grew in 80mg water per litre of kerosene and, after four weeks incubation, the concentration of water increased more than ten fold. Fungal influenced corrosion has been reported for carbon steel and aluminium alloys exposed to hydrocarbon fuels during transport to storage [13]. Rosales *et al.* [14] demonstrated metal ion binding by fungal mycelia resulting in metal ion concentration cells on aluminium surfaces. Differential aeration caused by the adherence of fungal mats can cause crevice corrosion. DeMele *et al.* and Videla *et al.* [15-16] reported that corrosivity increased with contact time due to accumulation of metabolites under fungal colonies attached to metal surfaces. De Maybaum *et al.* [17] and De Schiapparelli *et al.* [18] demonstrated the metabolic products enhanced aqueous phase aggressiveness even after the lifecycle of *Cladosporium sp.* completed.

White-rot fungi possess extracellular systems that are effective for the degradation of lignin. Earlier studies have shown that these fungi also may be effective degraders of recalcitrant hazardous organic molecules such as polycyclic

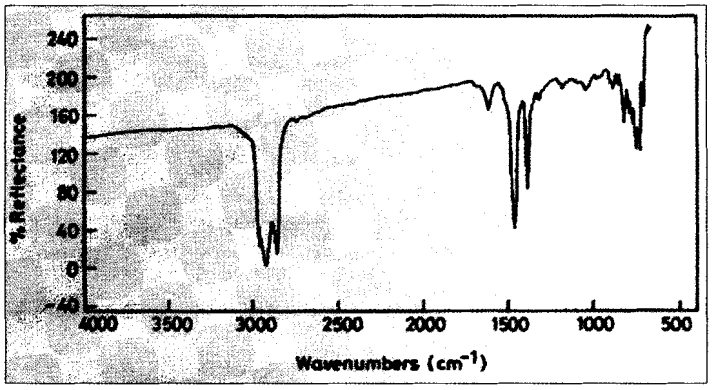

Fig.1. FTIR spectrum of diesel.

aromatic hydrocarbons (PAHs) [19-20]. Biocorrosion studies involving the use of natural individual species obtained from industrial systems are scarce; however, such studies would better address the actual problem, and to increase the understanding of the microbial species involved in microbial corrosion and their interactions with metal surfaces will be the basis for the development of new approaches for the detection, monitoring, and control of microbial corrosion in industrial facilities. The present study has been aimed to find out the nature of biodegradation of diesel and its effect on corrosion of mild steel.

Experimental methods

Sample collection and identification of fungal isolates

Diesel was collected by sterilized conical flasks from a diesel storage tank in SW India and transferred in an icebox from the site to the CECRI microbiology laboratory. One ml of the diesel sample was taken out from the sterilized conical flask and it was serially diluted using 9ml and 99ml sterile 1% peptone water broth, up to 10^{-9} dilutions and plated in potato dextrose agar by the pour-plate technique. Identification was based on the visual observation of isolates grown on the potato dextrose agar (PDA) plates, micromorphological studies in slide culture [21] at room temperature, and the taxonomic keys described in Hoog and Guarro [22].

Fugal culture propagation condition

The fungal cultures were first propagated in potato dextrose broth (PDB) media for three days on a rotating shaker. *Penicillium sp.*, *Candida sp.*, and *Aspergillus sp.* were incubated at incubated at 30°C on a rotary shaker (at 200rpm) for five days. Then they were grown on shredded cardboard (30mm long x 3mm wide) supplemented with a nutrient solution for two weeks. Conical flask (of 5l capacity), each containing 125g of shredded cardboard mixed with 500ml of nutrient solution and sterilized at 121°C for 30min, were inoculated with 5ml of the PDB propagated cultures. The nutrient solution had the following constituents:

KH_2PO_4	5.0g/l
$(NH_2)_2SO$	10.0g/l
$MgSO_4.7H_2O$	0.3g/l
$CaCl_2$	0.5g/l
yeast extract (*Himedia*)	0.5g/l

Trrace element stock solutions with the following composition were added to the media (1.0ml/l):

$FeSO_4.7H_2O$	1.3µg/l
$MnSO_4.H_2O$	3.8µg/l
$ZnSO_4.7H_2O$	3.5µg/l
$CoCl_2.6H_2O$	6.7µg/l

The degradation process

Erlenmeyer flasks containing 100ml of the potato dextrose broth (Hi-media, Mumbai, infusion from white potatoes: 200gm/l; dextrose: 20gm/l) were supplemented with 1gm of diesel and inoculated with fungal strains (5mm disks obtained from the outer edge of an actively growing culture) *Penicillium sp.*, *Candida sp.*, and *Aspergillus sp.* The flasks were incubated at 30°C for 20 days in an orbital shaker (at 150rpm). An uninocculated control flask was incubated in parallel to monitor abiotic losses of the diesel substrate.

Corrosion study

Mild steel (API 5LX grade) coupons of size 2.5 x 2.5cm were mechanically polished to mirror finish and then degreased using trichloro ethylene. In the present study, 500 ml of diesel with 2 % water containing 120 ppm chloride has been used as the control system, while 500 ml diesel with 2% of water containing 120 ppm chloride and inoculated with 5 ml of fungal inoculated systems such as *Penicillium sp.*, *Candida sp.*, and *Aspergillus sp.* were employed (System-II III & IV) used as the experimental system. After different immersion periods (of 5, 10, 15, and 20 days), the coupons were removed and pickled in pickling solutions, washed in water, and dried by using air drier. Final

weights of the six coupons in each system were taken, and the average corrosion rates were also calculated. The standard deviation for each system was also presented. A computer controlled XRD system was used to scan the corrosion products between 10° and 85° – 2θ with copper Kα radiation (Ni filter) at a rating of 40KV, 20mA. The dried corrosion products were collected and crushed into a fine powder and used for XRD analysis for determining the nature of oxides present in the corrosion product.

Chemical characterization

At the end of the 20-day incubation period, the residual diesel of the entire flask was extracted with an equal volume of dichloromethane. Evaporation of solvent was carried out in a hot water bath at 40°C. The resultant solution was analyzed by Fourier transform infra-red spectroscopy (FTIR) and nuclear magnetic-resonance spectroscopy (NMR). The FTIR spectrum was taken in the mid IR region of 400–4000cm^{-1} with 16-scan speed. The samples were mixed with spectroscopically-pure KBr in the ratio of 1:100 and the pellets were fixed in the sample holder, and the analysis was carried out. Infra-red peaks localized at 2960 and 2925cm^{-1} were used to calculate the CH_2/CH_3 ratio (absorbance) and functional group of both aliphatic and aromatic components present in diesel. Bruker (200-mHz) NMR analysis was used to detect the protons of the nuclei in the diesel compound. The sample of diesel was dissolved using deutrated chloroform solvent. Tetramethyl silane (TMS) was used as a reference standard.

Result and discussion

Microbiologically-influenced corrosion is one of the well-documented phenomena in corrosion, and causes deleterious effects on petroleum product pipelines, storage tanks, and in various industries. Moreover, diverse groups of bacteria and fungi have been reported in hydrocarbon degradation [23-26]. Water is needed for all forms of life and the availability of water influences the distribution and growth of micro-organisms. In addition to water, all organisms require carbon, nitrogen, phosphorous, sulphur, and other trace elements for growth. Most fungi are aerobes and are only found in aerobic habitats. Fungi grow in aquatic environments where they assimilate organic material and produce organic acids such as oxalic, lactic, formic, acetic, and citric acids.

Penicillium sp., *Cephalosporium sp.*, *Candida sp.*, *Mucor sp.*, *Aspergillus sp.*, and *Aspergillus niger* fungal strains were isolated from locally-available diesel. The products of microbial oxidation of hydrocarbons are alcohols, aldehydes, and aliphatic acids [1]. Several authors have reported a decrease in bulk pH due to metabolites produced during growth of fungi [7, 27]. De Mele *et al.* [15] demonstrated a correlation between growth of *Cladosporium sp.* and pH at fuel/water interfaces and measured pH values between 4 and 5 in the bulk medium. But nobody has explained the degradation pathway and its role on corrosion by fungi.

Identification of fungal isolates

Based on macroscopic and microscopic morphological characters, these strains were identified as belonging to the genus *Penicillum sp.*, *Candida sp.*, and *Aspergillus sp.* The genera *Aspergillus* and *Penicillium* belong to the phylum *Ascomycota*, found to utilize aromatic and aliphatic hydrocarbons as growth substrates [28-30].

Chemical characterization

FTIR spectroscopy of pure diesel shows that characteristics peaks at 2954cm^{-1}, 2852cm^{-1} and 2921cm^{-1} (C-H aliphatic stretch) presented in Fig.1. A peak at 1605cm^{-1} indicates C = C stretch in aromatic nuclei. The peaks at 1456cm^{-1} and 1377cm^{-1} indicate the CH aliphatic stretch for methyl groups. The peak at 809cm^{-1} indicates the disubstituted benzene, and at 698cm^{-1} indicates meta-disubstituted benzene. The spectrum for diesel with *Candida sp.*. shows (Fig.2a) a free OH peak at 3248cm^{-1}, and CH peaks at 2954cm^{-1}, 2921cm^{-1}, and 2852cm^{-1}. The peak at 1607cm^{-1} indicates the presence of C = C aromatic nuclei.

The peaks at 1463cm^{-1} and 1367cm^{-1} indicate the CH def in methyl group. The peak at 809cm^{-1} indicates the presence of disubstituted benzene. The aromatic compound peak could not be disturbed in the presence of *Candida sp.*, and shows that it would not be able to degrade aromatic compounds.

The spectrum for diesel with *Penicillium*

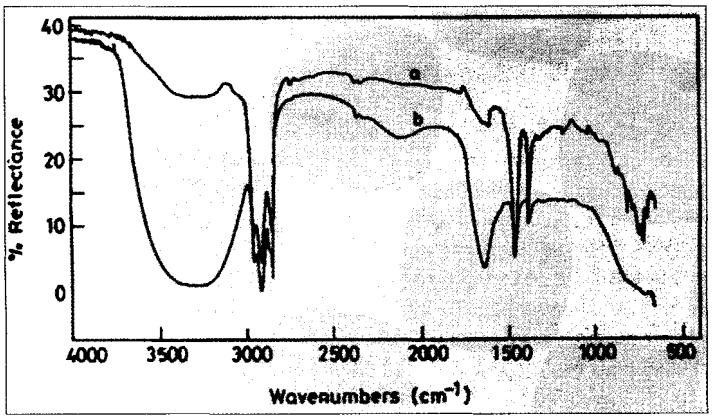

Fig.2. FTIR spectra:

a. Candida sp.
b. Penicillium sp.

sp. shows (Fig.2b) a free OH peak at 3324cm^{-1} and CH aliphatic stretch peaks at 2954cm^{-1}, 2922cm^{-1}, and 2852cm^{-1}, and C = C conjugated diene at 1634cm^{-1}. The CH def in methyl group is present at 1462cm^{-1} and 1376cm^{-1}. The spectrum for diesel with *Aspergillus sp.* shows (Fig.3) a free OH peak at 3315cm^{-1}. The peaks at 2954cm^{-1}, 2921cm^{-1}, and 2852cm^{-1} indicate the CH aliphatic stretch; C = C conjugated diene was noticed at 1633cm^{-1}. The peaks at 1457cm^{-1} and 1377cm^{-1} indicate the CH def in methyl group. In pure diesel, the peak at 809cm^{-1} was not observed in *Penicillium sp.* and *Aspergillus sp.*; the disubstituted benzene (809cm^{-1}) peak also was not be observed. It can be explained that the aromatic compounds are utilized by these strains. The FTIR results are supported by the NMR spectrum which, for pure diesel, shows (Fig.4a) some major peaks at 0-3 chemical shifts (δ), indicating the presence of aliphatic protons. Another peak at 6-7 chemical shift (δ) indicates the presence of benzene. In the presence of fungal species, the new peaks mostly occurred at 4-5 chemical shifts (δ), indicating the aliphatic protons (-CH_2-CH_2-)$_n$ are converted to (-O-CH_2-)$_n$ groups [31]. This shows that the addition of oxygen with carbon atoms, due to fungal metabolic activity. The common mechanisms for all strains are as follows:

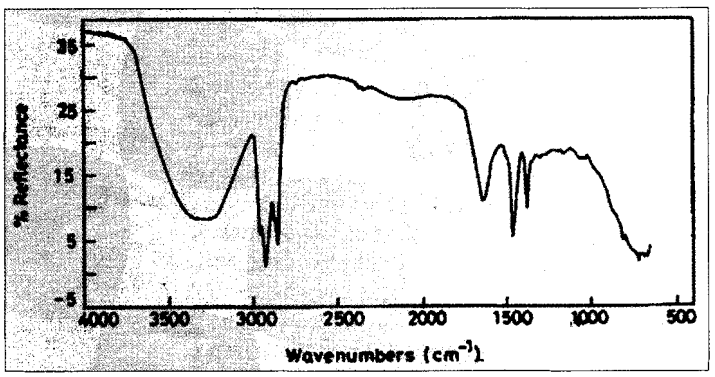

Fig.3. FTIR spectrum of Aspergillus sp.

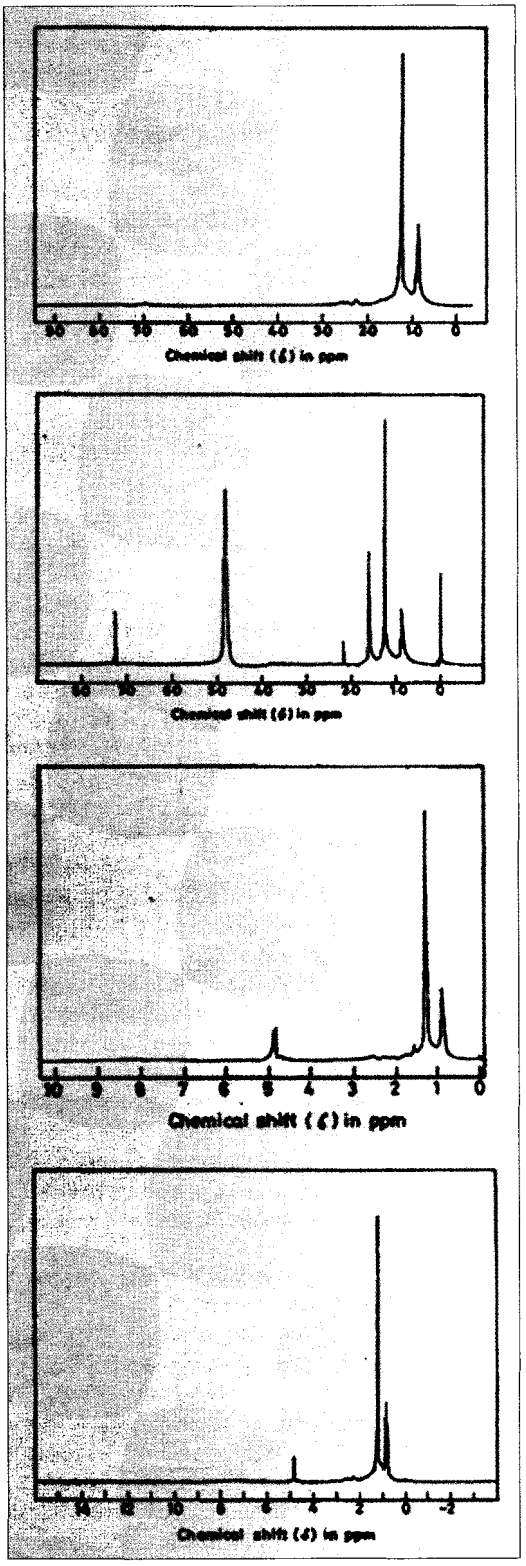

Fig.4. 1H NMR analysis of the degraded diesel (top-bottom):

a. pure diesel
b. Penicillium sp.
c. Aspergillus sp.
d. Candida sp.

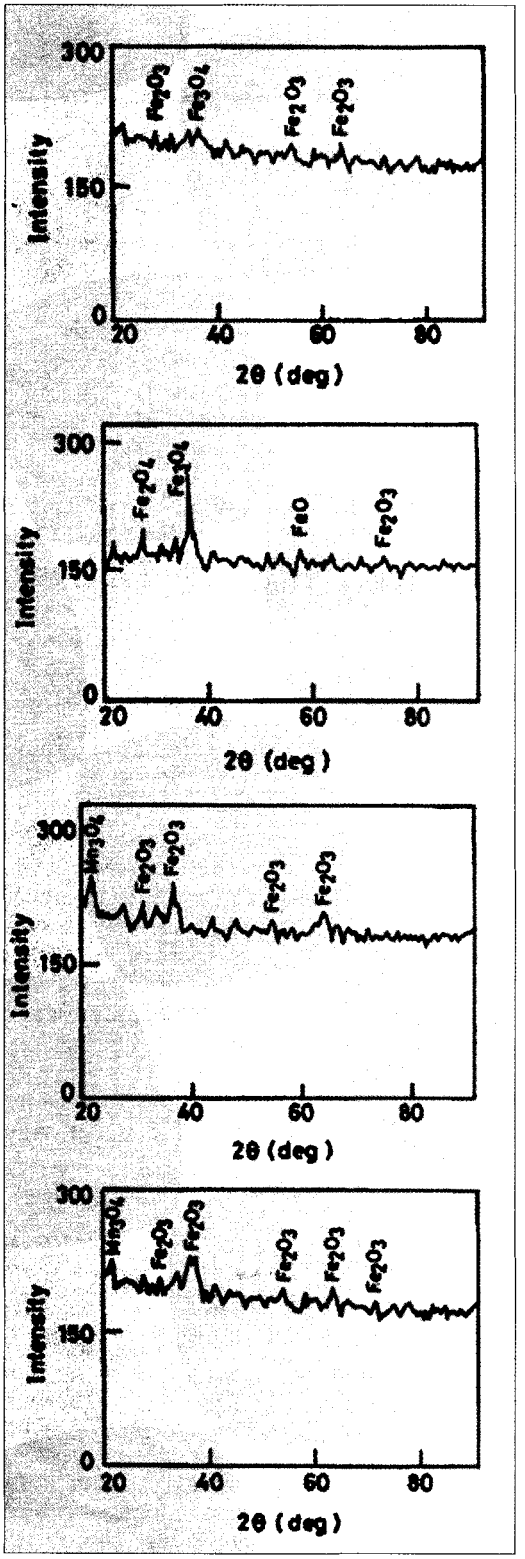

Fig.5: XRD patterns of corrosion product of various systems (top-bottom):

a. pure diesel
b. Penicillium sp.
c. Aspergillus sp.
d. Candida sp.

$$(\text{-}CH_2\text{-}CH_2\text{-})_n \xrightarrow[\text{Metabolism}]{\text{Fungal}} (\text{-}O\text{-}CH_2\text{-})_n$$

In the presence of *Penicillium sp.* (Fig.4b) the production of the (-O-CH_2-) group is greater when compared to *Aspergillus sp.*. (Fig.4c) and *Candida sp.* (Fig.4d). It can be concluded that the greater oxygen addition (i.e. consumption of hydrogen) is the reason for this. In pure diesel there is not much weight loss, but in fungal-inoculated diesel, the corrosion rate is high. The growth of three white-rot fungi in soil contaminated with polycyclic aromatic hydrocarbons, degradation by the fungal species was noticed by Anderson *et al.*[32].

Hammel *et al.* [33] reported an oxidative degradation of phenanthrene by the ligninolytic fungus *Phanerochaete chrysosporium* and degradation of pyrene by white-rot fungi [34]. Three strains of white-rot fungi, namely *Phanerochaete chrysosporium*, *Pleuroteus ostreatus*, and *Coriolus versiocolor* have been tested for their ability to degrade oil in contaminated soil. These fungi, which utilized lignin as an energy source, possess the ability to degrade the oil [35]. Peroxidases are a group of iso-enzymes that, with the H_2O_2, produced by the white-rot fungi, catalyse the oxidativative dopolymerization of lignin. Nitrogen is one of the nutrients that trigger ligninolyte enzyme production to degrade the pollutant by the white-rot fungi [36]. Generally, bacteria and fungi utilize the carbon sources from the petroleum products [37-39] and degrade the diesel.

Muthukumar *et al.* [31,40] reported the degradation of diesel in the presence of microbes, and noticed the role of degradation on corrosion. But in fungus, the high concentrations of carbon are suggested to induce the peroxidase enzymes production in fungal strains and thus can positively affect the degradation rates [41-42]; subsequently, the final pH may be dropped in the fuel and water interface.

Corrosion study

The weight-loss data for mild steel in pure diesel with 2% sodium chloride solution (system I) and diesel with 2% sodium chloride along with various fungal strains (systems II, III, and IV) are presented in Table 1.Corrosion rates are lower in the

Systems	No of days	Weight loss (mg)	Corrosion rate (mm/y)
System I 500ml diesel + 2% water (120ppm chloride)	5 10 20	0.033 ± 0.02 0.043 ± 0.04 0.061 ± 0.03	0.2369 0.1543 0.1095
System II 500ml diesel + 2% water (120ppm chloride) + *Pencillium sp.*	5 10 20	0.090 ± 0.02 0.120 ± 0.04 0.180 ± 0.03	0.6462 0.4308 0.3231
System III 500ml diesel + 2% water (120ppm chloride) + *Aspergillus sp.*	5 10 20	0.053 ± 0.02 0.062 ± 0.04 0.076 ± 0.03	0.3805 0.2226 0.1364
System IV 500ml diesel + 2% water(120ppm chloride) + *Candida sp.*	5 10 20	0.047 ± 0.02 0.056 ± 0.04 0.068 ± 0.03	0.3375 0.2010 0.1220

Table 1. Corrosion rate of mild steel API 5LX.

control system in the range between 0.1095 mm/y and 0.2369 mm/y. But in diesel with fungal inoculated system, the corrosion rate was much higher, in the range between 0.3231mm/y and 0.6462 mm/y in the presence of *Penicillum sp.*, while the corrosion rate of *Aspergillus sp.* was about 0.3805 mm/y, and that of *Candida sp.* was about 0.3375 mm/y.

Figure 5 (a-d) shows the XRD data for the fungal inoculated and its control system. The corrosion peaks were identified from the *Penicillum sp.*, *Aspergillus sp.*, and *Candida sp.* inoculated system; ferric oxides and ferric manganese complex are noticed. The high intensity of Fe_2O_3 peaks have been observed in the *Penicillium sp.* inoculated system (Fig.5b) when compared to the *Aspergillus sp.* and *Candida sp.* using X-ray diffraction. It can be concluded that the initial corrosion products of Fe^{++} combine with the H_2O_2 formed during fungal metabolic activity, and form Fe_2O_3. The possible following reaction might be the reason for the corrosion:

$$Fe \longrightarrow Fe^{++} + 2e^-$$

$$4Fe^{++} + 3H_2O_2 \longrightarrow 2Fe_2O_3 + 6H^+$$

$$6H^+ + 6e^- \longrightarrow 6H^+$$

Based on the overall result, we put forward the following hypothesis. It can be concluded that the converted organic compounds in diesel accelerate the formation of ferric oxide; subsequently, the fungi encourages the corrosion process by the formation of Fe_2O_3. Degraded carbon (diesel) acts as a good nutrient for fungi, which increases the proliferation of the fungal load on the coupon, and determines the nature of degradation and corrosion. Hence, the corrosion rate and nature of corrosion product can be seen to depend upon the fungal metabolic activity during diesel degradation.

Conclusion

The following conclusions have been made, which will be useful for the oil industry:

1. This study has provided a first look at the role of fungi in the biodegradation of diesel oil and in the corrosion process in petroleum products pipelines and storage tanks. It can be concluded that fungi is the most proficient organism involved in industrial diesel degradation: it is a major aromatic and aliphatic degrader that breaks the benzene ring and aliphatic to (-O-CH_2-), allowing inclusion of oxygen and accelerating the corrosion by ferric oxide formation in a diesel environment.

2. The selected strains of *Penicillium sp.*, *Aspergillus sp.*, and *Candida sp.* were subjected the degradation of diesel by FTIR analysis. The FTIR data reveals that *Penicillium sp.* quickly degrade the diesel when compared to *Aspergillus sp.* and *Candida sp.* The NMR data reveals tha the aliphatic hydrogen atom is consumed by the degrading fungal species and carbon-hydrogen bond is cleaved to carbon-oxygen bond. The present study explains that *Penicillium sp.* easily degrades the diesel when compared with *Aspergillus sp.* and *Candida sp.*

3. Even though, these bacteria could be useful in the bioremediation of diesel-polluted habitats, their presence in diesel pipeline and transportation facilities would lead to the reduction in the quality of diesel and, in turn, to economic loss.

4. Microbial corrosion can be avoided by selection of good inhibitors and biocides.

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