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Bacterial degradation of naphtha and its influence on corrosion

A. Rajasekar, S. Maruthamuthu *, N. Muthukumar, S. Mohanan, P. Subramanian, N. Palaniswamy

Corrosion Science and Engineering Division, Central Electrochemical Research Institute, Karaikudi 630006, India

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Abstract

The degradation problem of naphtha arises since hydrocarbon acts as an excellent food source for a wide variety of microorganisms. Microbial activity leads to unacceptable level of turbidity, corrosion of pipeline and souring of stored product. In the present study, biode-gradation of naphtha in the storage tank and its influence on corrosion was studied. The corrosion studies were carried out by gravimetric method. Uniform corrosion was observed from the weight loss coupons in naphtha (0.024 mm/yr) whereas in presence of naphtha with water, blisters (1.2052 mm/yr) were noticed. The naphtha degradation by microbes was characterized by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR). IR study reveals the formation of primary alcohol during degradation process. It was found that microbes degrade ($-CH_2--CH_2-$)_n to $R--CH_3$. Iron bacteria, manganese oxidizing bacteria, acid producers, and heterotrophic bacteria were enumerated and identified in the pipeline. SRB could not be noticed. Since water stratifies in the pipeline, the naphtha-degraded product may adsorb on pipeline, which would enhance the rate of microbial corrosion. On the basis of degradation and corrosion data, a hypothesis for microbial corrosion has been proposed.

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Keywords: Mild steel; Microbiological corrosion; Naphtha degradation; IR spectroscopy; NMR spectroscopy; Weight loss; XRD

^{*} Corresponding author. Tel.: +91-4565-227550; fax: +91-4565-227779. *E-mail address:* marutha_m@yahoo.com (S. Maruthamuthu).

1. Introduction

Microbial contamination of fuels has been the cause of intermittent operational problems throughout the world for many years. Even less then 0.1% of water is enough for microbial activity leading to biodegradation of hydrocarbons. In order to prevent the effects of microbial growth, several lines of approach such as good house keeping practices, treatment with biocides to limit the growth and use of special tank linings, etc. are used. The types and ability of microorganisms to degrade petroleum hydrocarbons have been widely documented [1-6]. The corrosion of carbon steel in oil-in-water under hydrodynamic was studied by Becerra et al. [7]. The influence of inhibitors on the rate and mechanism of corrosion in petroleum product in presence of moisture was studied by various investigators [8–11]. Internal corrosion as a cause for leakage of steel tanks has been documented in US, France, Sweden and Switzerland by various sources [12–14]. Jana et al. [15] carried out a failure analysis study in oil pipelines at Mumbai offshore and concluded that the combined effect of CO₂, SRB, and chloride in the low velocity area caused the severe corrosion and failure of pipeline. Muthukumar et al. [16] reported the degradation of diesel in presence of microbes and noticed the role of degradation on corrosion. But no literature is available on the mechanism of the microbial corrosion in naphtha pipeline. Recently CECRI has noticed severe corrosion problem in a naphtha pipeline at Southwest India. The length of the pipeline was 5.5 km and corrosion products about 10 kg were collected from the pipeline every two months. Large quantity of sludge was noticed in the naphtha storage tank where the disposal of sludge had to be cleared by Pollution Control Board. The microbial growth in the sludge often causes severe turbidity and cloudiness of naphtha. Moreover, sludge often changes the actual chemical properties of naphtha in the storage tank and in transporting pipelines. In the present study, the nature of degradation of naphtha in a pipeline and its effect on corrosion have been assessed and discussed.

2. Materials and methods

2.1. Bacterial enumeration in corrosion product and in sludge

By using sterilized conical flasks, samples of naphtha and corrosion products from the filters and sludge from storage tanks were collected. These samples were transported by using icebox from sites to CECRI, Karaikudi. The collected samples were serially diluted (10-fold) using 9 ml of sterile distilled water-blanks and the samples were plated by pour plate technique. The nutrient agar medium, iron medium, API Broth and Mn-medium were used to enumerate heterotrophic bacteria, iron bacteria, sulphate reducing bacteria and manganese depositing bacteria respectively. The collected samples were serially diluted up to 10^{-6} dilution. One millilitre (1 ml) of each sample was poured into sterile petridishes. The prepared respective sterile medium was also poured into petridishes. The plates were gently swirled so that the medium might be distributed evenly in the plate. Plates in triplicate were prepared for each dilution. The plates were inverted and incubated at room temperature for 24 h. After 24 h the colonies were counted. The plates containing bacterial colonies with 30–300 numbers were selected for calculation. The bacterial colonies were expressed as colony forming units per millilitre (CFU/ml) of water. Morphologically dissimilar colonies were selected randomly from all plates and isolated colonies were purified using appropriate medium by streaking methods. The pure cultures were maintained in specific slants for further analysis. The isolated bacterial cultures were identified by their morphological and biochemical characteristics. Sixteen genera were identified in the sludge samples. The strains were maintained at 40 °C to keep the microbial strain viable. The isolated bacterial cultures were identified up to genus level by their morphological and biochemical characterization viz gram staining, motility, indole, methyl red, voges-proskauer test, citrate test, H₂S test, carbohydrate fermentation test, catalase test, oxidase test, starch, gelatin, lipid hydrolysis, etc. [17].

2.2. Chemical characterization

Five grams (5 g) sample of sludge was mixed with 100 ml of triple distilled water and agitated for 2 h using shaker. After shaking, the samples were filtered and the filtrates were used for chloride and sulphate analysis. Chloride and sulphate were also estimated in contaminated water collected in the storage tank. Chloride was estimated by Mohr's method and sulphate was estimated by the gravimetric method. FTIR was used for the analysis of the biochemical characteristics of the sample of sludge. The spectrum was taken in the mid IR region of $400-4000 \text{ cm}^{-1}$. The spectrum was recorded using ATR (attenuated total reflectance) technique. The sample was directly placed in the zinc selenide crystal and the spectrum was recorded in the transmittance mode. Bruker (300 mHz) NMR Spectroscopy was used for the analysis of the sludge samples. The sample of sludge was dissolved using deutrated chloroform solvent. Tetra Methyl Silane (TMS) was used as a reference standard. A computer controlled XRD system, JEOL Model JDX-8030 was used to scan the corrosion products (collected from filters) between 10° and 85° -2θ with copper K α radiation (Ni filter) at a rating of 40 KV, 20 mA. Corrosion products collected at the filters and sludge collected from storage tank were dried and crushed to a fine powder and used for XRD analysis to determine the nature of the complex formed in the corrosion products and in sludge.

2.3. Corrosion studies

Mild steel (API 5LX grade) coupons of size 2.5×2.5 cm were mechanically polished to mirror finish and then degreased using trichloro ethylene. In the present study, 500 ml of naphtha has been used as the control system, while 500 ml naphtha with 2% of water (collected from the naphtha storage tank) was used as the experimental system. Moreover, 500 ml of water alone has also been used as another control for weight loss studies. After seven 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 deviations for each system are presented.

3. Results and discussion

Microbial activity in oil industries can result in fuel contamination, unacceptable level of turbidity, filter plugging, corrosion of storage tanks, pipelines and souring of stored products [18–21]. Hence, it is quite essential to investigate the nature of degradation. The degradation of diesel and crude oil has been studied in oil spilled soil by Delille [22]. Lloyed Jones and Trudgill [23] isolated alicyclic hydrocarbon utilizing consortia *Rhodococcus* sp., *Flavobacterium* sp. and *Pseudomonas* sp. isolated from oil refinery soil. April [24] noticed 64 species of elemental fungi from five flare pits in northern and western Canada that were tested for their ability to degrade crude oil using gas chromatography analysis which indicated that the species were capable of degrading hydrocarbon of the aliphatic fraction of crude oil, nC_{12} , nC_{26} . Besides, Roffey [25] reported on the aerobic and anaerobic degradation in crude oil and in diesel storage tanks. In the present study, the roles of microbes on degradation of naphtha and its effect on corrosion have been explained.

3.1. Microbial analysis

Table 1 shows the data on different types of bacterial count (heterotrophic bacteria, iron bacteria, acid producers, manganese oxidizing bacteria, sulphate reducing bacteria) in corrosion products collected from filters, water-naphtha interface, water and sludge collected from storage tanks. It can be seen from the data given in the Table 1 that the heterotrophic bacteria is in the range between 10^6 and 10^7 CFU/ml, iron bacteria is in the range between 10³ and 10⁵ CFU/ml and manganese oxidizers are in the range between 10^3 and 10^5 whereas sulphate reducing bacteria is nil. Among the heterotrophic bacteria isolated, gram negative bacteria seems to be more dominant than gram positive bacteria by 80%. Generic distribution was found to be Pseudomonas sp. (20%), Bacillus sp. (10%), Gallionella sp. (10%) and Vibrio sp. (10%). All the manganese-depositing bacteria isolated from the sample of naphtha sludge were completely dominated by gram-negative bacteria. Generic composition was dominated by Gallionella sp. (25%) and followed by Legionella sp. (12.5%) and Siderocapsa sp. (12.5%). Iron bacteria isolated from the sample of naphtha sludge was completely dominated by gram negative bacteria. Among them, Gallionella sp. (22.22%) and Thiobacillus sp. were equally shared and followed by Bacillus sp. (11.11%). All the bacteria were found to be gram negative. Among them *Thiobacillus* sp. is shared by 28.6% followed by Thiospira 14.25% and Sulfolobus sp. 14.25%. In all the type of microbes, gram-negative bacteria completely dominated. It indicates that gram-negative bacteria are more active in degradation of naphtha.

Table 1

Bacterial population in (Panel 1) water/naphtha-water system and sludge, (Panel 2) corrosion product samples and sludge

Sl. no.	Collection point	System	Total viable count (TVC)				
	I · · ·		HB (CFU/ml)	IB (CFU/ml)	MOB (CFU/ml)	AP (CFU/ml)	SRB (CFU/ml)
Panel 1							
1	Storage tank-1	Water-naph- tha interface	6.20×10^{7}	4.90×10^{5}	6.70×10^{5}	3.80×10^{5}	NIL
2	Storage tank-2	Water	4.80×10^{7}	6.30×10^5	4.20×10^{5}	1.02×10^{4}	NIL ^a
Panel 2							
1	Corrosion j filter-1	products from	1.28×10^{6}	8.20×10^{3}	1.36×10^{4}	1.02×10^{4}	NIL
2	Corrosion j filter-2	products from	1.68×10^{6}	6.10×10^{5}	4.20×10^{3}	9.70×10 ⁵	NIL
3	Sludge		7.20×10^6	1.12×10^{4}	1.62×10^4	3.80×10^{3}	NIL ^a

HB = heterotrophic bacteria, IB = iron bacteria, MOB = manganese oxidizing bacteria, AP = acid producing bacteria, SRB = sulphate reducing bacteria.

^aOne tube blackening at 10⁻² dilution.

3.2. Chemical characterization

Table 2 presents the data of sulphate and chloride concentrations present in the water and corrosion product sample collected at two sites. The chloride content in water was in the range from 7 to 175 mg/l whereas sulphate content was in the range from 155 to 198 ppm. The adsorption of chloride content in the corrosion product was about 7 ppm and the sulphate was about 25 ppm at both the sites. The presence of chloride indicates the chances of water being present in the corrosion product. The sources of sulphate in water could be assumed that it would have come from the sulphur content of naphtha through oxidation process by acid producers. It is surprised that though water has some sulphate, SRB could not be noticed in water and in corrosion product. Oxidation of elemental sulphur results in the formation of sulphate and hydrogen ions and sulphur oxidation characteristically results in

Table 2

Chloride and sulphate concentrations in water samples/sludge/corrosion products collected at storage tanks

Location and sample	Chloride (mg/l)	Sulphate (mg/l)
Storage tank-1 water	7	155
Storage tank-2 water	175	198
Sludge collected from storage tank-2	12	60
Corrosion products from filter-1	7	26
Corrosion products from filter-2	7	23

lowering of pH. The pH range for growth of SRB is 6.5–8.5 with optimum being 7.2– 7.5 [26]. The broad ecological classes of sulphur-oxidizing bacteria can be discerned, among those living at neutral pH and those living acid pH [27]. Many of the forms are living at acid pH. In the present study, the pH of the sludge was 6.8. In the interface between naphtha and water, the pH was about in the range between 5.5 and 6.0. The absence of SRB may be due to the domination of acid producers (AP), iron oxidizing bacteria (IOB) and manganese oxidizing bacteria (MOB). The acidity creates "syntrophy" for the three communities of microbes namely acid producers, manganese oxidizers and iron oxidizers which suppress the proliferation of SRB. *Pseudomonas* sp., *Bacillus*sp., *Gallionella* sp., *Thiobacillus* sp., *Thiospira* sp., *Sulfolobus* sp., *Legionella* sp., and *Siderocapsa* sp. were noticed in the sludge.

The FTIR spectrum (Fig. 1a) of the naphtha peaks at 2955, 2923, 2855 cm⁻¹ indicate the presence of CH-aliphatic stretch. The peaks at 1457 and 1378 cm⁻¹, indicate the CH def for methyl group. The peaks in the range between 693 and 727 cm⁻¹ indicate the presence of substituted benzene. The FTIR spectrum (Fig. 1b) of sludge shows a broad peak between 3000 and 3500 cm⁻¹ indicating the presence of OH-band. Another peak at 1033 cm⁻¹ indicates the CO stretching for primary alcohol group. A peak at 1635 cm⁻¹ indicates the presence of C=C conjugated diene. The ¹H NMR spectrum of naphtha shows (Fig. 2), peak at 0–3 chemical shifts (δ) indicating the presence of aliphatic protons in naphtha compounds. The other peak at 7 chemical shifts (δ) indicates the presence of aromatic nuclei in naphtha compounds, where as in the sludge (Fig. 3) the aromatic peak (7 chemical shifts δ) could not be noticed. Sharp peak at 7.26 chemical shifts (δ) was confirmed



Fig. 1. FTIR spectrum of naphtha degradation: (a) naphtha and (b) sludge.



Fig. 2. ¹H NMR spectrum of naphtha.



Fig. 3. ¹H NMR spectrum of sludge.

as solvent peak in the sludge. Fig. 3 shows, a peak at 1.5 chemical shifts (δ) indicating the presence of water. Another broad peak at 4–6 chemical shift indicates the presence of heteroatom-included proton (hydrogen). This may be due to the adsorption of S—H peak. The presence of elemental sulphur in naphtha is also supporting the S—H peak. Naphtha is not a single compound it has many organic constituents viz *n*-butane, 2-methylbutane, 2-methylpentane, *n*-hexane, benzene, *n*-heptane, *n*-methylcyclohexane, *n*-methylbenzene, 3-methylheptane, 3,5-dimethylcycloheptane and *n*-nonane with sulphur (Table 3). IR results reveal that the CH-aliphatic stretch was degraded by microbes. The absence of peaks in the sludge sample in the range between 674 and 727 indicates that benzene ring is consumed

Name of the constituents	Percentage by weight	
<i>n</i> -Butane	2.85	
2-Methylbutane	4.65	
2-Methylpentane	2.71	
<i>n</i> -Hexane	3.21	
Benzene	2.33	
<i>n</i> -Heptane	10.45	
<i>n</i> -Methylcyclohexane	15.96	
<i>n</i> -Methylbenzene	14.43	
3-Methylheptane	3.11	
3,5-Dimethylcycloheptane	2.13	
<i>n</i> -Nonane	4.64	
Sulphur	0.04	

Table	3		
Major	constituents	in	naphtha

by microbes since the major components of *n*-heptane $(-CH_2-CH_2-)_n$, toluene and benzene in naphtha are degraded as $R-CH_3$ by consumption of hydrogen. The hydrogen is utilized for ATP synthesis by microbes. It reveals that $R-CH_3$ is converted to $R-CH_2-OH$ (primary alcohol) by the addition of oxygen. On the basis of heterotrophic bacteria and autotrophic bacterial physiology, it can be assumed that heterotrophic bacteria utilizes energy from naphtha and acid producing bacteria converts elemental sulphur in naphtha into thiosulphate which can be formed as complex in the sludge. Since naphtha with water is transported through the pipeline under pressure the supply of oxygen may be sufficient for the aerobic bacteria. The concentration of chloride was about 10 ppm in the water and in the sludge samples. This data reveals that chloride contribution on corrosion may be nil. Heterotrophic bacteria break the organic constituents by consumption of hydrogen and convert into R-CH₃ whereas autotrophic bacteria increases the addition of oxygen and convert into R-CH₂-OH (primary alcohol). The addition of oxygen or sulphate (4–5 chemical shifts (δ)) heteroatom can be seen in NMR spectrum and spectrum indicates that both aliphatic and aromatic group are degraded by microbes. The addition of oxygen is due to very rapid addition of oxygen by Gallionella sp. and Legionella sp. which supports with the observation made by Muthukumar et al. [16]. Moreover Gallionella sp. being chemolithotrophic, microaerophilic and acidophilic species, produces toxic oxygen products (H_2O_2) as an outcome of its metabolism, it needs the scavenging mechanism to overcome the toxic product. Here the role played by manganese oxidizing bacteria and iron bacteria seems to be vital. The manganese and iron oxidizers were used for scavenging the produced toxic oxygen product. This is where the syntrophic relationship between Thiobacillus sp. and Gallionella sp. could be appreciated. Mars et al. [28] studied the effect of trichloro ethylene on the competitive behaviour of toluene-degrading bacteria viz Pseudomonas putida and Burkholderia sp. Besides Shim [29] also reported the trichloroethylene degradation by toluene-o-xylene monooxygenase of *Pseudomonas* sp. Microbial degradation of nitrobenzene was

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reported by Zhao and Ward [30] by Comamonas testosterone and Acidovorax delafieldii and noticed the broad degradation ability towards nitrobenzene. Zhu et al. [31] characterized the microbial communities like *Proteobacteria* sp. and *Coma*monas denitrificans in gas industry pipelines and mentioned about the importance of microbially influenced corrosion. In composite crude oil [32] where the aqueous and oil phases co-exist, the potentially limited bacterial corrosion may be enhanced in a matrix population of crude oil degraders and non degraders. Even where the corrosion causing bacteria do not utilize hydrocarbon as energy sources, the intermediate degradation will boost the energy available to the corrosion causing bacteria to sustain the corrosion reaction. Jobson et al. [33] also reported that intermediate hydrocarbon degradation products make available energy sources for the physiological activities of the corrosion bacterium *Desulfovibrio* sp. This supply of utilizable hydrocarbon degradation products explained why was corrosion intense in the Pembiana oil pipeline. Besides Westlake et al. [34] reported corrosion by ferric reducing bacteria isolated from oil production system at Pembiana oil pipeline. They identified oil degraders viz Bacillus, Aeromonas, Cornebacteria in the oil wells and suggested that heterotrophic bacteria converted oil into lactic acid which was utilized by *Pseudomonas* and *Clostidium* and encouraged the formation of FeS. In Indian refined diesel product pipeline, the benzene in the diesel was utilized by heterotrophic bacteria Brucella sp. and converted into aliphatic compound [16] which supported the theory by Traxler and Flamming [35] whereas Gallionella sp. took energy from only aliphatic compounds in diesel. Toluene and ethyl benzene were used as sources of carbon and energy by microbes where as the ethyl benzene was degraded by monooxygenase enzyme [36]. Besides Rhodococcus rhodochrons S-2 produces extra cellular polysaccharides that help to live in aromatic fraction [37].

3.3. Corrosion studies

Table 4 shows the weight loss data for various systems. In presence of water, the weight loss was 26 mg, whereas in the naphtha–water system the weight loss was 201 mg. In presence of naphtha, the weight loss was 40 mg. Blisters were noticed within 24 hours on mild steel coupons in the system of naphtha + water. In water and naphtha systems, uniform corrosion was noticed (Fig. 4). The formation of blistering indicates the adsorption of elemental sulphur from the naphtha, which acts as

contosion studies for various systems					
Sl. no.	System	Weight loss (mg)	Corrosion rate (mm/yr)	Form of corrosion	
1	Water (storage tank)	26 ± 2	0.1579	Uniform	
2	500 ml naphtha	40 ± 3	0.2405	Uniform	
3	500 ml naphtha + 2% water	201 ± 2	1.2052	Blisters	

Table 4 Corrosion studies for various systems



Uniform corrosion

Blisters with sludge formation

Fig. 4. Uniform corrosion and Blisters mild steel coupons in the system of naphtha and naphtha + water system respectively.



AP – Acid producers, IOB – Iron oxidizing bacteria, MOB – Manganese oxidizing bacteria

Fig. 5. Schematic illustrations of blister formation on API5LX.

cathode and parent metal acts as anode. Due to the presence of water, the conductivity might be more, which initiates the formation of blisters on the coupons within 24 h. It can be assumed from the result that the adsorption of sulphur species in presence of contaminated water initiate corrosion whereas microbes beneath the blisters accelerate the corrosion process. The formation of corrosion cell has been presented in Fig. 5. It reveals that sulphur initiates corrosion when bacteria accelerates the corrosion. Black sludge formation has also been noticed on the coupon. Fig. 6 shows the quantity of corrosion product collected from filters in the field. The weight of corrosion product was higher during the months of November and December. It indirectly indicates that the presence of higher quantity of water (rainy season) in naphtha may accelerate the corrosion when compared to other seasons. The electrochemical reaction between sulphur and parent metal is enhanced by conductivity of water which supports with the observation made by Kennelley et al. [38] in sour gas pipeline.



Fig. 6. Corrosion products collected from filters at various months.

3.4. XRD analysis of corrosion products/sludge samples

Figs. 7 and 8 present the details of XRD data corresponding to the phases present in the corrosion product sample and sludge collected at different sites. Ferric oxide, silicon-di-oxide, manganese and its oxides were observed in the corrosion product samples. α Iron III oxide hydroxide, Iron sulphate and ferric sulphate were noticed in the sludge. The presence of ferric oxide and manganese oxide indicate the role of iron/manganese bacteria on the formation of corrosion product/sludge on the pipeline. XRD results reveal the presence of ferrous and ferric sulphate in the sludge which indicate the role of iron/manganese bacteria while acid producers cause the formation of sulphate in the water and sludge. In the inner side of the tank severe corrosion was noticed, where there is no paint on the inner surface of the tank. The paints peeled off from the surface at many spots and this severe corrosion may be due to the abrasion caused by the movement of the floating rooftop followed by exposure of the surface to the atmosphere. In can be concluded that Fe²⁺ comes from the



Fig. 7. XRD spectra of the corrosion product sample from filters.



Fig. 8. XRD spectra of the sludge sample.

storage tank/pipes and combined with organic degraded products whereas organic compound is degraded by heterotrophic bacteria and Fe^{2+} can be converted as Fe^{3+} by autotrophs viz. *Gallionella* sp. and *Legionella* sp., which can be noticed in XRD data [16].



The addition of oxygen or sulphate (4–5 def) heteroatom was also seen in NMR spectrum. It can be understood that the formation of corrosion product from the inside storage tank/pipeline combines with naphtha degraded product on the metal surface and settles down in the storage tank as sludge. A simplest model can be envisaged. The present observation indicates that degradation starts from naphtha–water interface and it can be understood that the presence of inorganic products (iron oxide and iron sulphate) from the storage tank combine with naphtha degraded products in the interface and settles down in the storage tank as sludge. Since sulphur acts as cathode, acidity is formed on the metal surface which creates syntrophy for the autotrophs viz. acid producers, manganese oxidizers and iron oxidizers. It can be concluded that due to the formation of acidity, sulphate reducing bacteria (SRB) could not be noticed in the pipeline. The aromatic and aliphatic compounds in naphtha are degraded by heterotrophic bacterial activity and subsequently autotrophic bacteria converts ferrous and manganese on the metal into oxides.



HB – Heterotrophic bacteriaAP – Acid producersIOB – Iron oxidizing bacteriaMOB - Manganese oxidizing bacteriaATP – Adenosine triphosphate

4. Conclusions

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

- 1. Uniform corrosion was noticed in water collected from storage tank as well as in pure naphtha whereas blister formation was seen in the naphtha with water combination. The blister formation is due to the cathodic species of sulphur. It can be inferred that presence of sulphur initiates the corrosion process. Hence, sulphur should be eliminated to avoid the initiation of corrosion.
- 2. The presence of manganese and ferric ions in corrosion product/sludge indicates the role of manganese oxidizers and iron oxidizers on corrosion and degradation of naphtha.
- 3. Since water stratifies on the pipeline, the degraded naphtha product would enhance the microbial corrosion. It can also been concluded that water contamination should be avoided to prevent corrosion.
- 4. The investigation clearly indicates the possibility of break down of naphtha by heterotrophic bacteria. Even though, these bacteria could be useful in the

bioremediation of diesel polluted habitat, their presence in naphtha storage and transportation facilities would lead to the reduction in the quality of naphtha and in turn economic loss. The microbial corrosion can be avoided by selection of good inhibitors/biocide.

5. The present study also suggests that storage tank design may be changed to avoid water contamination.

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