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Role of *Brucella* sp. and *Gallionella* sp. in oil degradation and corrosion

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Abstract

Microbiologically influenced corrosion is responsible for most of the internal corrosion in oil transmission pipelines and storage tanks. In the present study, the role of bacteria on oil degradation and its influence on corrosion have been studied. Two systems (biotic and abiotic) with and without inorganic content and bacteria were employed for studying degradation and corrosion. The aerobic heterotrophic bacterial population (HB) was found to be higher in the presence of inorganic medium than its absence. The oil degradation by microbes was characterized by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). The corrosion studies were carried out by gravimetric method. It was found that *Gallionella* sp. degraded aliphatic protons $-CH_2-CH_2-$ to $-O-CH_2-$ whereas *Brucella* sp. converted only aromatic ring to aliphatic protons. The following inferences have been made from this study: (a) inorganic contents in contaminated water determine the oil degradation in storage tanks and transporting pipelines; (b) the degraded product may adsorb on pipeline, which would enhance the rate of microbial corrosion.

Keywords: Bacteria; Inorganic nutrients; Diesel degradation; Corrosion; FTIR; NMR; XRD

1. Introduction

Microbial contamination of fuels has been the cause of intermittent operational problems throughout the world for many years where water is essential for biodegradation, whereas less than 0.1% is enough for microbial activity. Microbial activity can result in fuel contamination, filter plugging, and corrosion of tanks and pipelines and souring of stored products. In order to prevent the effects of microbial growth several lines of approach are used, namely good house keeping practices, treatment with biocides to limit growth and the use of special tank linings, etc. The types and ability of microorganisms to degrade petroleum hydrocarbons have been widely documented [1–5]. The importance of internal corrosion as a cause for leakage of steel tanks has been documented by various sources [6]. A comprehensive US Environmental Protection Agency reports documented 6–10% of tanks failures were caused by internal corrosion [7]. In France, it was estimated by a major oil company that 10% of underground storage tanks leaked due to internal corrosion while other data from France indicated 8.5% of the leakages were caused by internal corrosion. Switzerland has reported 5% of its tank leakages are caused by internal corrosion [6]. Sweden had reported that half of its leaking tanks are due to internal corrosion [8]. But no literature is available on mechanisms of microbial corrosion in oil pipelines along with oil degradation.

The present study has been carried out to find out the nature of degradation of oil in a pipeline and its effect on corrosion. The bacterial species used in this study were collected from a petroleum pipeline at North West India.

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2. Materials and methods

2.1. Sampling area and procedure

Sludge samples were collected using sterilized conical flasks at various pumping station of a petroleum product pipelines, North West of India. The samples were transported by icebox from various sites to CECRI-Microbiology laboratory. Various types of bacteria viz. heterotrophic bacteria, iron bacteria, acid producers and manganese depositors were enumerated and identified. Two identified genera *Gallionella* sp. *Brucella* sp. were chosen for the current diesel degradation and corrosion studies.

2.2. Experimental work of the degradation process

The medium used for detecting the oil degrading process by bacteria was Bushnell–Hass broth (di-potassium hydrogen phosphate 4.74 g/l; potassium hydrogen phosphate 0.56 g/l) (Hi-Media, Mumbai) and Bushnell–Hass agar. Two sets of bottles were used for the diesel degradation studies using the selected bacterial strains. To one set of the bottle, 5 ml of each of the bacterial strains, 10 ml of diesel and 100 ml of media were added. Total viable count inoculated was enumerated by serial dilution in each set of bottles on the first day. A control was identically maintained but without the inoculum. To another set of bottles, 5 ml of each bacterial culture and 100 ml of media was added. No diesel was added. The total viable count inoculated on the first day was enumerated in each set of the bottles. All the bottles were maintained at room temperature for an inoculation period of 15 days, after which the hydrocarbon utilizing bacterial population was enumerated. 1 ml of sample was poured into sterile petridishes. The prepared respective sterile medium was poured into petridishes. The plates were gently swirled so that the medium spreads evenly in the plate. Triplicate plates were inverted and incubated at 37 °C for 24 h. After 24 h the colonies were counted. The plates containing bacterial colonies with 30–300 numbers were selected and colony counts per ml was calculated and average values are presented. The bacterial colonies are expressed as colony forming units per ml (CFU/ml).

2.3. Confirmatory test for diesel degrading bacterial strains

To confirm the diesel degrading effect by the strains, after the 15th day incubation period, one loopful of each culture from respective bottles were taken and streaked on BH agar plates and were incubated for about 15 days at 37 °C and the results were observed for growth.

2.4. Extraction of diesel and analysis of the degraded diesel

After incubation, the degraded samples were separated for analytical purpose using a separating funnel. Much care was taken to see that the extracted oil was a clear solution without any water content. The extracted oil was characterized by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR). The FTIR study spectrum was taken in the mid IR region of 400–4000 cm^{-1} with 16-scan speed. The samples were mixed with spectroscopically pure KBr in the ratio of 1:100, pellets were fixed in the sample holder, and the analyses were carried out. NMR analysis was used to detect the protons of the nuclei in the compound. The volume of injected sample was about 20 μl. Deutrated chloroform was used as a solvent and tetra methyl silane (TMS) as reference standard.

2.5. Corrosion study

Mild steel (API 5LX grade) specimens of one inch square were polished to mirror finish mechanically and degreased using trichloro ethylene. To find the role of inorganic nutrients in oil environment on corrosion process, this study has been carried out in 500 ml of oil with 2% of water (120 ppm of chloride) as control system. 500 ml oil with 2% of water and inorganic medium (Bushnel–Hass broth) was used as experimental system. In both the systems, 2 ml of mixed cultures with an initial load of 2.2×10^6 were inoculated for weight loss study. After the inoculation period of 14 days, the coupons were removed and pickled in pickling solution, washed in water and dried by using air drier. Final weights of the coupons were taken and the corrosion rate has been calculated. The average weight loss values of six coupons are presented.

2.6. XRD study

A computer controlled XRD system, JEOL Model JDX-8030 was used to scan corrosion product (sludge) samples between 10° and 85° -2θ with copper Kα radiation (Ni filter) at a rating of 40 kV, 20 mA. Corrosion product collected from coupon studies were dried and crushed to a fine powder and used for XRD analysis to determine the nature of the complex formed on the coupons in laboratory study.

3. Results and discussion

The degradation problem arises where excellent food sources (hydrocarbon fuels) for a wide variety of microorganisms are allowed to remain at stimulate

temperatures, in contact with water. 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 [9]. Hence, it is quite essential to investigate the nature of degradation and its control. The degradation of diesel and crude oil has been studied in oil-spilled soils by Delille [10]. Lloyed Jones and Trudgill [11] isolated alicyclic hydrocarbon utilizing consortia *Rhodococcus* sp., *Flavobacterium* sp. and *Pseudomonas* sp. isolated from oil refinery soil. April [12] noticed 64 species of filamentous fungi from five flare pits in Northern and Western Canada that were tested for their ability to degrade crude oil using gas chromatographic analysis. Gas chromatography indicated that species were capable of hydrocarbon degradation of the aliphatic fraction of crude oil, $n\mathrm{C}_{12}$-n-C_{26}, and degradation of compounds that in the aromatic fraction was not observed. Besides, Roffey [13] reported the aerobic and anaerobic degradation of crude oil/refined products of diesel in storage tanks. In oil pipelines, water can stratify at the bottom of the line if the velocity less than the required to entrain the water and sweep it through the system which encourage degradation in transporting pipeline also. Recently Jana [14] noticed a failure analysis in oil pipelines at Mumbai offshore. They suggested that the combined effect of CO_2, SRB, and chloride in the low velocity area caused the severe corrosion and failure of pipeline.

Generally bacteria from trehalolipids, rhamnolipids, or analogous structures as constituents of their cell walls in which hydrocarbons are dissolved and transported to the cytoplasmic membrane. *Acinetobacter* strains have been shown to excrete particle with the outer membrane, the hydrocarbons can be transferred to the membrane. Thus the general strategy is to produce and emulsify that mediates transfer of the hydrocarbon from the oil layer to the cytoplasm membrane [15]. In the present study, role of some microbes on diesel degradation and its effect on corrosion has been studied.

3.1. Total viable count in presence and absence of oil with BH medium

Tables 1 and 2 portrays the changes in bacterial population in presence and absence of diesel with Bushnell–Hass medium. Bacterial load in BH broth supplemented with diesel over a period of 15 days was recorded and presented in Table 1. From this, it is evident that all the two bacterial cultures were able to utilize/degrade diesel resulting in corresponding increase in population. Irrespective of the initial load all the two bacterial inoculants exhibited 10-fold increase in the population density at the end of the experimental period (15th day). On the other hand, examining the data presented in Table 2 clearly reveals the inability of bacterium to survive in BH broth without the supplementation of diesel as all of them exhibited remarkable load reduction.

Table 1
Change in bacterial population in diesel supplemented BH broth

Bacterial strains	Duration	
	1st day (CFU/ml)	15th day (CFU/ml)
Gallionella sp.	3.02×10^7	3.22×10^8
Brucella sp.	3.12×10^6	3.12×10^7

CFU, colony forming units.

Table 2
Changes in bacterial population in BH broth

Bacterial strains	Duration	
	1st day (CFU/ml)	15th day (CFU/ml)
Gallionella sp.	2.8×10^6	2.72×10^4
Brucella sp.	2.78×10^6	2.85×10^3

3.2. FTIR analysis of diesel degradation

The IR spectroscopy of pure diesel shows the characteristics bands at 2954, 2923 and 2854 cm^{-1} (C—H aliphatic stretch); 1604 and 1557 cm^{-1} (C=C stretch in aromatic nuclei); 781, 699 (meta disubstituted benzene) and 810 cm^{-1} (disubstituted benzene). In the control system, the spectrum shows the bands of BH medium with diesel (without bacteria) at 2954, 2923 and 2854 cm^{-1} (C—H aliphatic stretch); 1604 and 1463 cm^{-1} (C=C aromatic nuclei); 781 and 699 cm^{-1} (meta substituted benzene); and 810 cm^{-1} (disubstituted benzene). While adding *Gallionella* sp. CH aliphatic stretch, C=C aromatic nuclei, conjugated diene were observed. Besides, it can be noticed that the presence of transmittance at 1456 and 1377 cm^{-1} are higher when compared to control. In FTIR spectrum of *Brucella* sp., the observed peaks were OH group, CH aliphatic stretch, conjugated diene, and CH def in methyl and meta disubstituted benzene.

The spectrum of pure diesel indicates the presence of carbon and hydrogen stretching bond and carbon hydrogen def in methyl. While adding bacterial strains a new peak mostly occurred at 1633 cm^{-1} which can be identified as C=C. The aromatic nuclei peaks disappeared because of degradation. It can be assumed that C=C aromatic bonds were broken by *Brucella* sp. and converted as aliphatic C=C stretching bond. In the presence of *Gallionella* sp. benzene degradation could not be observed. The low percentage of transmittance in *Brucella* sp. at 1633 cm^{-1} reveals the formation of aliphatic bond strength higher than *Gallionella* sp. So, it can be assumed that the *Brucella* sp. prefers aromatic than aliphatic chains.

3.3. ^1H NMR analysis of diesel degradation

The ^1H NMR spectrum of pure diesel shows some major peaks at 0–3 chemical shifts (δ). It indicates the presence of aliphatic protons. The presence of another peak at 6–7 chemical shifts (δ) indicates the presence of benzene ring. In the presence of *Gallionella* sp. the benzene ring peak (6–7 chemical shifts (δ)) could not be disturbed. But in the presence of *Brucella* sp., benzene ring peak at 6–7 chemical shifts (δ) value could not be noticed which supports the FTIR data. The spectrum for *Brucella* sp. shows the absence of —O—CH_2— formation at 4–5 chemical shifts (δ) values. It can be explained that there is no conversion of aliphatic hydrocarbons. It reveals that the benzene ring is utilized by *Brucella* sp. and converted to aliphatic compounds, which support the theory proposed, by Traxler and Flannery [16].

The new peaks obtained at 4–5 chemical shifts (δ) after the degradation by *Gallionella* sp. indicate the addition of oxygen with carbon atoms. So the present study reveals that aliphatic hydrogen was consumed by degrading bacteria and C—H bond was cleaved into C—O bond. From the NMR spectrum, it can be explained that the addition of oxygen is the common mechanism for the strains. *Gallionella* sp. produced more (—O—CH_2—) groups. This is due to very rapid addition of oxygen by this strain, which means that large amount of hydrogen and carbon group is consumed by *Gallionella* sp. when compared to *Brucella* sp. The soil fungus *Cladophialophora* sp. was also capable to degrade alkylated benzenes (toluene, ethyl benzene, xylene) by a combination of assimilation and cometabolism. Toluene and ethyl benzene were used as sources of carbon and energy. The ethyl benzene was degraded by monooxygenase enzyme [17]. Besides *Rhodococcus rhodochrons* S-2 produces extra cellular polysaccharides that helps to live in aromatic fraction [18]. Since *Gallionela* sp. oxidizes ferrous ions into ferric oxide in presence of oxygen, it utilizes energy from carbon and hydrogen during oil degradation. Besides, it can be assumed that *Brucella* sp. help for the formation of more aliphatic protons in diesel which may be utilized by *Gallionella* sp.

$$(-CH_2-CH_2-)_n \xrightarrow[\text{Bacterial Metabolism}]{Fe^{2+}\rightarrow Fe^{3+}} (-O-CH_2-)_n$$

3.4. Corrosion study

Table 3 shows the corrosion rate of steel in presence of mixed culture with and without inorganic nutrients. In presence of inorganic nutrients corrosion rate was lower (0.0923 mmpy) than control system (0.3580 mmpy). The presence of bacteria was higher in oil and on coupons (Fig. 1) in presence of inorganic nutrients when compared with control. It indicates that the presence of inorganic content with degraded compounds of oil encourages the attachment of bacteria on coupon and oil. Adsorption of phosphate/nitrate from inorganic nutrients may reduce the corrosion rate of steel. The present study reveals that the adsorbed film (iron phosphate with degraded carbon content) will accelerate corrosion in later stage [19]. Inorganic nutrients and degraded carbon act as good nutrients for bacteria, which increases the proliferation of bacteria on coupon and determine the nature of degradation and corrosion.

Table 3
Corrosion study for inorganic nutrients + 120 ppm chloride and 120 ppm chloride alone

System	Wt. loss (mg)	Corrosion rate (mmpy)
Control + 500 ml oil + 2% water (120 ppm chloride) + 1 ml mixed culture	149.6 ± 15.6	0.3580
Test: 500 ml oil + 2% water (inorganic nutrient + 120 ppm chloride) + 1 ml mixed culture	38.0 ± 1.1	0.0924

3.5. XRD study

Fig. 2 presents the XRD data corresponding to the phases present in the corrosion product sample. Ferric

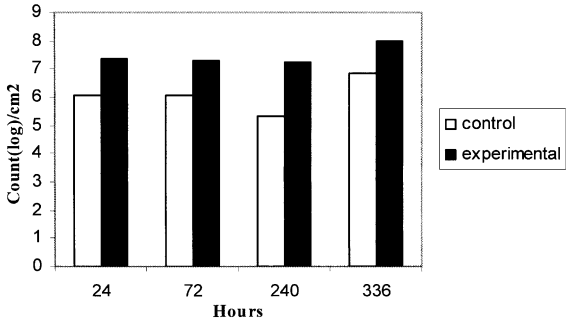


Fig. 1. Enumeration of mixed bacterial population on coupon.

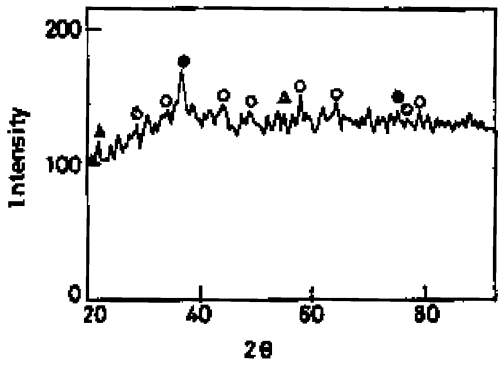
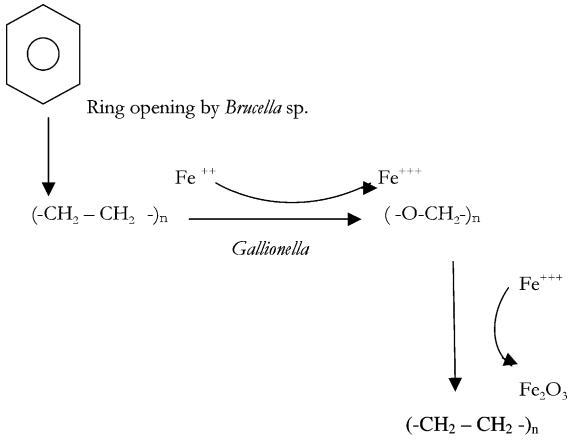


Fig. 2. XRD analysis of corrosion product (sludge) samples: ○, Fe_2O_3; ●, FeO; ▲, Fe_2SiO_4; △, Fe_2O_4.

silicate, iron manganese complex, ferric oxide, ferrous chloride and ferrous oxide were noticed in presence of mixed cultures of *Brucella* sp. and *Gallionella* sp. The present study explains that *Brucella* sp. converts aromatic ring to aliphatic protons which would be utilized by *Gallionella* sp. Subsquently the iron bacteria *Gallionella* sp. converts ferrous to ferric which combines with oxygen to form as ferric oxide that is the major phase as corrosion product. On the basis of the present study the following model has been proposed:



4. Conclusions

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

1. *Brucella* sp. is the major aromatic degrader, which breaks the benzene ring to aliphatic.
2. *Gallionella* sp. helps for the addition of oxygen and converts only aliphatic group and accelerates corrosion by ferric formation.
3. The quantity of inorganic content also determines the oil degradation and microbiologically influenced corrosion on materials.
4. This investigation clearly indicates the possibility of breakdown of diesel by bacterial strains. Even though, these isolates could be useful in the bioremediation of diesel polluted habitat, their presence in diesel storage and transportation facilities would lead to the reduction in the quality of diesel and in turn economic loss. More experiments need to be carried out with different microbes to find the microbial corrosion in oil pipeline.

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