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Role of cationic and nonionic surfactants on biocidal efficiency in diesel-water interface

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Abstract

Biodegradation occurs at the interface between diesel and water. The microbial contamination can result in inhibitor/fuel degradation that leads to the unacceptable level of turbidity, filter plugging, corrosion of storage tanks, pipeline and souring of stored products. Hence, selection of biocides/inhibitors is an important aspect in petroleum product transporting pipeline. Three biocides (cationic and nonionic) were employed to study the biodegradation of diesel in diesel–water interface. The biocidal efficiency on biodegradation of diesel was examined using Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR) and gas chromatography mass spectrometry (GC–MS). Polyoxyethyleneglycol dodecyl ether [BRIJ-35] and polyethylene glycol-*p*-isooctylphenyl ether [TRITON-X-100] had higher bactericidal efficiency than Dodecyl ethyl dimethyl ammonium bromide [DDAB]. But the cationic biocide (DDAB) gave good biocidal efficiency at the interface. The data are explained in terms of a model that postulates the formation of "micelle" at the diesel–water interface.

Keywords: Biocide; Cationic; Nonionic; Micelle; Biodegradation

1. Introduction

Microbiologically influenced corrosion (MIC) in oil pipelines is mainly associated with different types of bacteria and fungi [1–7]. MIC is responsible for most of the internal corrosion that leads to leaking of steel tanks, souring of fuels and failure of pipelines. A comprehensive US Environmental Protection Agency report documented that 6–10% of tank failures were caused by internal corrosion. Therefore the attention has been turned towards the control of microorganisms [8–11]. Hence, it is important to have knowledge of microbial problems occurring in the storage and pipelines and to develop methods or propose designs, so as to minimize oil degradation/failure of pipelines [12,13]. Jana [14] noticed a failure in an oil pipeline at Mumbai offshore. They suggested that the combined effect of carbon dioxide (CO_2), sulphate reducing bacteria (SRB) and chloride in the low velocity area cause the severe corrosion and failure of pipeline. Growth of many prokaryotic and eukaryotic microorganisms on hydrocarbons, often associated with the production of surface-active compounds, is a well-reported process [15–19]. In general, the degradation of hydrocarbons is accompanied by an emulsification, resulting in a greater oil–water interface [20]. These emulsifiers, which are generally extracellular, may be relatively simple glycolipids or complex high molecular weight substances, often of uncertain structure [21–24]. Their production allows the uptake and utilisation of hydrocarbons and this, in turn, leads to the growth of microbial cells, which has important implication on the oil industry [25]. In order to control the effects of microbial growth, several lines of approach were used viz., good house keeping practices, treatment with biocides to limit growth and the use of special tank linings, etc. [26]. The present authors feel that the bacteria should be killed at the interface, which will be useful to stop the production of emulsion [25,27,28] and diesel degradation. Thus identification of inhibitors/biocides which could act at the interface between diesel and water in petroleum transporting pipeline. Since the characteristics of biocides are not evaluated properly before use, many of the misapplication of biocides resulted in MIC [29,12]. Hence, selection and application of good biocide is needed in petroleum product pipelines. In the present investigation one cationic compound and two nonionic compounds have been selected to control the bacterial degradation process

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and the mechanism for biocidal action has been proposed along with solution chemistry.

2. Materials and methods

2.1. Sample collections and bacteria used

The strains *Serratia marcescens* ACE 2 and *Bacillus cereus* ACE 4 [30] were used in this study were isolated from oil transporting pipeline from a oil refinery in North west India (the nucleotide sequences data have been deposited in GenBank under the sequence numbers DQ092416 and AY912105).

2.2. Biocides employed

Three biocides, one cationic {Dodecyl ethyl dimethyl ammonium bromide [DDAB]} and two nonionic {polyoxyethyleneglycol dodecyl ether (polyoxyethylene-(23)-lauryl ether) [BRIJ-35]; polyethylene glycol-*p*-isooctylphenyl ether (octylphenoxy polyethoxy ethanol) [TRITON-X-100]} were employed to study their efficacy towards hydrocarbon utilizers.

2.3. Preparation of diesel-soluble biocides

0.1 g of respective biocide was dissolved in100 ml of solvent (ethylene glycol monobutyl ether) in a separate makeup flask to give a concentration of 1000 ppm and this was taken as stock solution. Ten milliliters of stock solution was added to 200 ml of BH broth, which gave a concentration of 50 ppm of diesel-soluble biocides.

2.4. Biodegradation study

The medium used for detecting the biodegradation process was Bushnell–Hass broth and Bushnell–Hass agar (BH, Hi-Media). BH medium contained, per liter: magnesium sulphate 0.20 g, calcium chloride 0.02 g, monopotassium phosphate 1 g, dipotassium phosphate 1 g, ammonium nitrate 1 g and ferric chloride 0.05 g. Seven sets of Erlenmeyer flasks were used for the biocidal efficiency studies using the mixture cultures. Six sets of Erlenmeyer flasks containing 200 ml of the BH broth, 50 ml diesel oil, 50 ppm of biocides each with mixed culture (*B. cereus* and *S. marcescens*) having an optical density of 0.045 at 600 nm (initial load about 2.1×10^9) were inoculated. In the absence of biocides, control flask was incubated parallelly to monitor biocidal efficiency. The flasks were incubated at 30 °C for 30 days in an orbital shaker (150 rpm). Total viable counts (TVC) were performed for seven sets of experimental systems after incubation of inocula at different time periods (5, 10, 15, 20, 25 and 30 days). The standard plating method was carried out for the enumeration of TVC and the colonies were counted after 48 h of incubation at 30 °C. During degradation, pH was also measured in the diesel–water interface for each system at different time intervals (5, 10, 15, 20 25 and 30 days).

2.5. Analytical methods

At the end of the 30 days of incubation period, the residual diesel was extracted with an equal volume of dichloromethane (DCM). Evaporation of solvent (DCM) was carried out in a water bath at 40 °C. The resultant solute of diesel samples (1 μl) was analyzed by employing FTIR NMR and GC–MS analysis. FTIR (Perkin-Elmer, paragon 500 model) was used to detect functional group of the compound. The spectrum was taken in the mid IR region of 400–4000 cm^{-1} with 16-scans. The samples were mixed with spectroscopically pure KBr crystal and the pellets were fixed in the sample holder and the spectrum was recorded in the transmittance mode. ^1H NMR (Bruker 300 MHz) was used to detect the protons of the nuclei in the compound. Deutrated chloroform was used as solvent. TMS (tetra methyl silane) was used as an internal reference standard. Twenty microliters of the sample was taken for analysis. The 1 μl of the resultant solute of diesel samples were analyzed by Thermo Finnigan gas chromatography/mass spectrometry (Trace MS equipped with a RTX-5 capillary column (30 m long × 0.25 mm i.d.) and high purity nitrogen as carrier gas). The oven was programmed between 80 and 250 °C at a heating temperature of 10 °C/min. The GC retention data of the inhibitor correspond to structural assignations done after NIST library search with a database and by mass spectra interpretation.

3. Results

3.1. Enumeration of bacteria during degradation

BH broth supplemented with diesel (without biocide addition as control) and their bacterial count was recorded at regular intervals of 5 days and the bacterial counts of the test samples (with biocides concentration of 50 ppm) were also recorded and compared with control and the results are shown in Fig. 1. Low recovery of bacterial population was recorded at the fifth day of incubation in all diesel soluble biocide. Exponential growth was observed in the control (without biocide addition). In diesel-soluble system, test sample (with biocides) shows initially a lower bacterial count than the control that is of about (4.7×10^4 CFU/ml). After 15 days of incubation, gradual

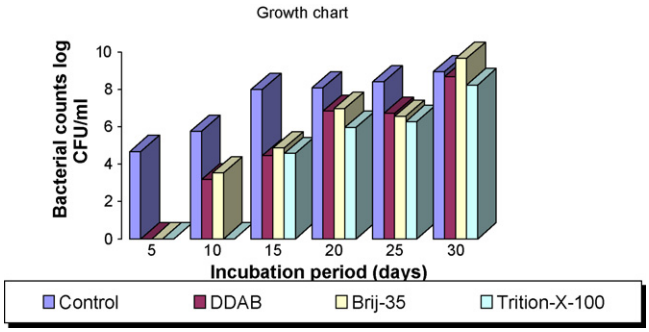

Fig. 1. Bacterial growth chart for in presence and absence of cationic and nonionic biocides. Control: without biocide; DDAB: dodecyl ethyl dimethyl ammonium bromide; BRIJ-35: polyoxyethyleneglycol dodecyl ether; TRITON-X-100: polyethylene glycol-*p*-isooctylphenyl ether.

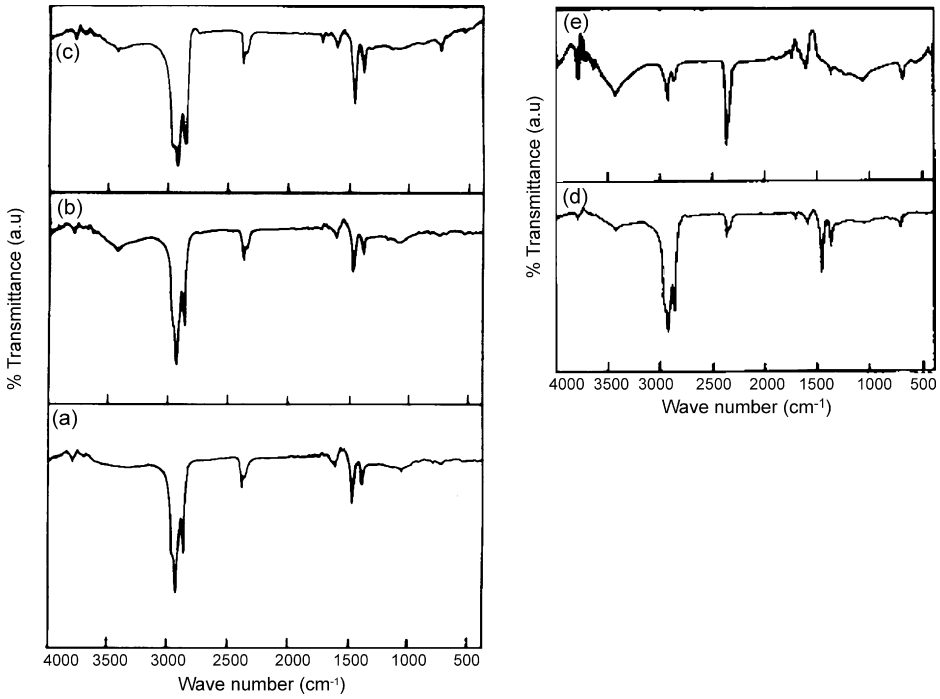

Fig. 2. FTIR spectrum of (a) pure diesel, (b) control (without biocide), (c) DDAB, (d) BRIJ-35 and (e) TRITON-X-100.

increase in the bacterial count was observed in presence of all biocide systems. There is no significant difference in killing efficiency between biocide systems and control. It reveals that the used biocides are effective upto 15 days and due to regeneration capability of bacteria, the high bacterial count was noticed after 30 days. It is evident that the bacterial isolates were able to utilize/degrade the diesel sample, which can be concluded with the corresponding increase in population with time.

The pH of BH broth is 7.0 ± 0.2. The initial pH of the test samples was nearly between 7.26 and 7.42 on the first day of incubation. On the thirtieth day of incubation, the pH of the control sample was noted as 7.75 and lowest pH value of 6.86 was noticed in BRIJ-35 addition system. Significant difference in pH could not be noticed.

3.2. FTIR analysis of diesel degradation

FTIR spectrum of pure diesel shows (Fig. 2a) the characteristics bands at 2951, 2919 and 2852 cm^{-1} (C–H aliphatic stretch); 1706 cm^{-1} (C=O carbonyl group); 1608, 1549 and 1459 cm^{-1} (C=C stretch in aromatic nuclei); 1375 cm^{-1} (C–H def. for methyl group); 1232 and 1092 cm^{-1} (C=O stretch for C–O–C alicyclic anhydride group); 808, 722 and 699 cm^{-1} (C–H stretch for substituted benzene).

In the control system (without biocide addition) the IR spectrum shows (Fig. 2b) the bands at 2925 and 2855 cm^{-1} (C–H aliphatic stretch); 1601 and 1460 cm^{-1} (C=C aromatic nuclei); 1376 cm^{-1} (C–H def. for methyl group); 1061 cm^{-1} (C–O stretch for alicyclic anhydride group).

IR spectrum of pure diesel with cationic biocide (DDAB) system shows (Fig. 2c) the characteristics band at 3783 cm^{-1} (NH stretch); 2925 and 2855 cm^{-1} (C–H aliphatic stretch); 1602 and 1460 cm^{-1} (C=C stretch in aromatic nuclei); 1376 cm^{-1} (C–H def. for methyl group); 724 cm^{-1} (mono substituted benzene).

IR spectrum of pure diesel with nonionic biocide (BRIJ-35) system shows (Fig. 2d) the characteristics band at 3422 cm^{-1} (OH Stretch); 2924 and 2859 cm^{-1} (C–H aliphatic stretch); 1726 (C=O carbonyl group); 1597 and 1434 cm^{-1} (C=C stretch in aromatic nuclei); 1361 cm^{-1} (C–H def. for methyl group); 1058 cm^{-1} (C–O stretch for C–O–C alicyclic anhydride group); 669 cm^{-1} (chloride peak).

IR spectrum of pure diesel with nonionic biocide (TRITON-X-100) system shows (Fig. 2e) the characteristics band at 3415 cm^{-1} (OH stretch); 2925 and 2855 cm^{-1} (C–H aliphatic stretch); 1718 cm^{-1} (C=O carbonyl group); 1601 and 1460 cm^{-1} (C=C stretch in aromatic nuclei); 1376 cm^{-1} (C–H def. for methyl group); 724 cm^{-1} (mono substituted benzene).

3.3. NMR analysis of diesel degradation

In all five solutions the NMR spectrum showed (Fig. 3a–e) characteristic aliphatic [0–3 chemical shifts (δ)], and aromatic [6–7 chemical shifts (δ)] protons. In the control solution however a new peak [3.5–4.5 chemical shifts (δ)] was noticed indicating the presence of oxygen; which could have resulted from the degradation of diesel by bacteria as per the following reaction.

$$-[CH_2{-}CH_2]- \xrightarrow[\text{degradation}]{\text{bacterial}} -[O{-}CH_2]_n$$

This extra peak did not show the presence of cationic biocide (DDAB) indicating that the degradation of diesel did not

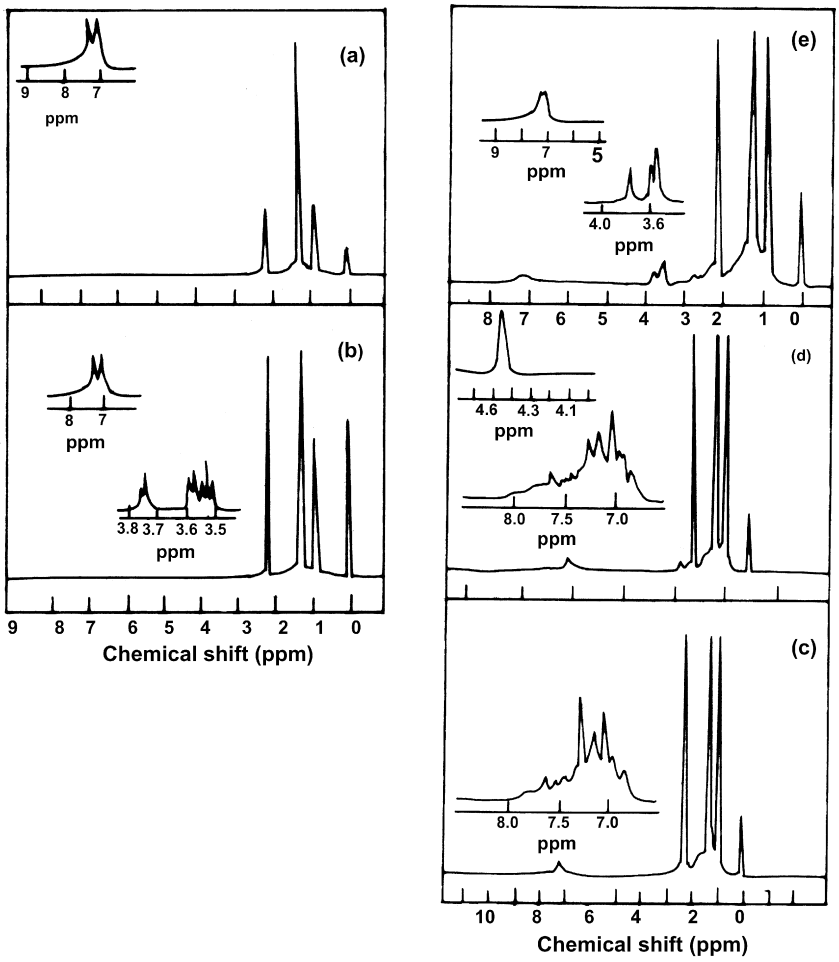

Fig. 3. NMR spectrum of (a) pure diesel, (b) control (without biocide), (c) DDAB, (d) BRIJ-35 and (e) TRITON-X-100.

occur. On the other hand appearance of the oxygen peak in the presence of both nonionic biocides (BRIJ-35 and TRITON X-100) indicate that these biocides could not control microbial activities.

3.4. GC–MS analysis of diesel degradation

The GC retention data of the diesel correspond to structural assignations done after NIST library search with a database and by mass spectra interpretation are presented. From the GC–MS analysis (Fig. 4a and Table 1), it is observed that the diesel (uninoculated system) consists of aliphatic and aromatic hydrocarbons.

In presence of bacterial cultures (ACE 2 and ACE 4) with pure diesel (Fig. 4b and Table 2), the compounds could not be observed at 0.75, 1.18, 5.40, 6.46, 8.20, 18.17, 19.31 retention time which indicate the bacteria utilizes these compounds as a food source. Cationic biocide (DDAB) with pure diesel system along with bacterial cultures (ACE 2 and ACE 4) (Fig. 4c and Table 3) shows the aliphatic and aromatic components, which is similar to the control system (without bacteria). There is no new degraded compound formation in presence of cationic biocide (DDAB). But in the presence of nonionic biocides (BRIJ-35 and TRITION-X-100) (Fig. 4d and e and Tables 4 and 5) the peaks could not be observed at 0.75, 1.18, 5.40, 6.46, 8.20, 18.17, 19.31 retention time which indicate that these biocides could not control microbial activities.

In presence of cationic biocide (DDAB), the peak reduction could not be noticed which is same when compared to the control system (Fig. 4a). But in the presence nonionic biocides [BRIJ-35 AND TRITON-X-100], the remarkable peak reduction could be noticed. Total seven compounds were consumed by bacterial species (ACE 2 and ACE 4). It reveals that bacteria consume these compounds for their metabolic activity (respiratory process). It is due to the nonionic biocides, which was not able to kill the microbes at diesel/water interface. Because nonionic biocides (BRIJ-35 and TRITON-X-100) does not have the micelle character.

4. Discussion

Microbial contamination of fuel has been the cause of intermittent operational problems throughout the world for many years and more recently, the frequency and severity of cases appear to be increasing dramatically [5]. Jobson [31] also reported that intermediate hydrocarbon degradation products

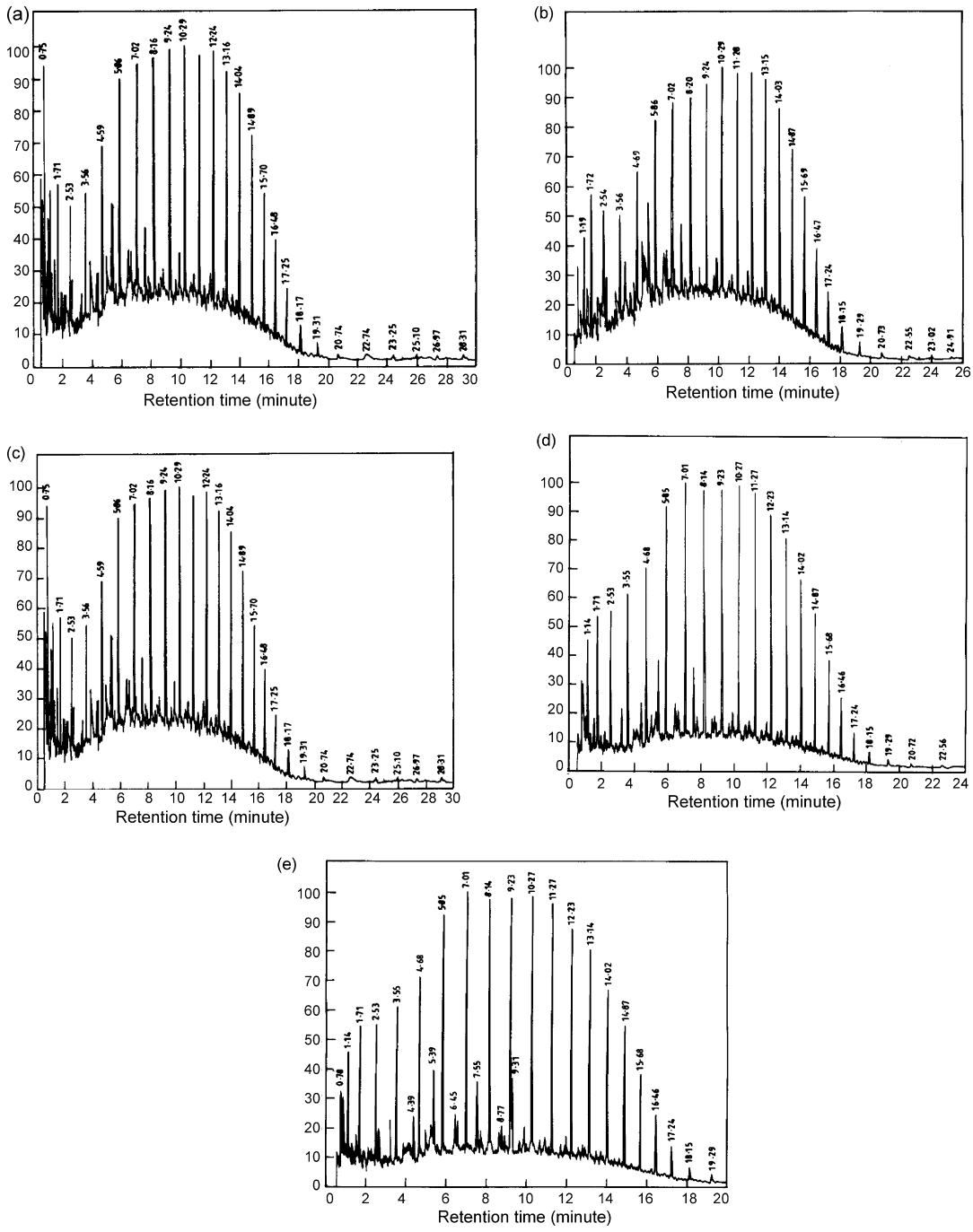

Fig. 4. GCMS spectrum of (a) pure diesel, (b) control, (c) DDAB, (d) BRIJ-35 and (e) TRITON-X-100.

make available as energy sources for the physiological activities of bacteria, the SRB (*Desulfovibrio* sp.) and explained the reason for MIC is intense in the Pembiana oil pipeline. Samant and Anto [32] reported SRB in oil pipeline and noticed the interaction between chloride ion and SRB on corrosion. A strain of SRB (*Desulfovibrio* sp.) was isolated from the microbial communities involved in microbiologically influenced corrosion in gas and oil transporting pipeline in the Gulf of Mexico by Jan et al. [33] and Mora-Mendoze et al. [34]. Hence, petroleum pipeline industries add preservatives/biocides to avoid the contamination or degradation of petroleum products. The selection, based on the mechanism of action of biocides in the water/oil interface is an important factor while procuring the biocides from market. Since pipeline industries are facing problems in the selection of inhibitors/biocides, the present study has been undertaken to investigate the behaviour of one cationic biocide (DDAB) and two nonionic biocides [BRIJ-35 and TRITON-X-100] on diesel degradation. In the present study, the role of micelle formation on killing effect of bacteria has also been investigated.

The FTIR studies reveal that the new peaks noticed at 1717 and 3400 cm^{-1} indicate the presence of carbonyl group and OH stretch peak in the nonionic biocides (TRITON-X-100 and

Table 1
GCMS data of pure diesel

Retention time	Compound	Molecular formulae	Molecular weight
0.75	*O*-dimethyl benzene	C_8H_{10}	106
1.18	Benzene-(1-methylethyl)	C_9H_{12}	120
1.71	Undecane	$C_{11}H_{24}$	156
2.53	Dodecane	$C_{12}H_{26}$	170
3.55	Tridecane	$C_{13}H_{28}$	184
4.69	Tetradecane	$C_{14}H_{30}$	198
5.40	Dodecane-2,2,4,9,11,11-hexamethyl	$C_{18}H_{38}$	254
5.86	Pentadecane	$C_{15}H_{32}$	212
6.46	Napthalene-1,6,7-trimethyl	$C_{13}H_{14}$	170
7.02	Hexadecane	$C_{16}H_{34}$	226
7.56	Octadecane-3-ethyl-5-[2-ethylbutyl]	$C_{26}H_{54}$	366
8.16	Heptadecane	$C_{17}H_{36}$	240
8.20	Pentadecane-2,6,10,14-tetramethyl	$C_{19}H_{40}$	268
9.24	Octadecane	$C_{18}H_{38}$	254
9.32	Dotriacontane	$C_{32}H_{66}$	450
10.29	Heneicosane	$C_{21}H_{44}$	296
11.28	Eicosane	$C_{20}H_{42}$	282
12.24	Octacosane	$C_{28}H_{58}$	394
13.16	Docosane	$C_{22}H_{46}$	310
14.04	Heptadecane-9-hexyl	$C_{23}H_{48}$	324
14.89	Tricosane	$C_{23}H_{48}$	324
15.70	Docosane	$C_{22}H_{46}$	310
16.48	Heptacosane	$C_{27}H_{56}$	380
17.25	Heptacosane	$C_{27}H_{50}$	380
18.17	Octadecane-3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$	366
19.31	Dotriacontane	$C_{32}H_{66}$	450

Table 2
GCMS data of pure diesel with bacterial cultures (ACE 2 and ACE 4)

Time	Compound	Molecular formulae	Molecular weight
1.71	Undecane	$C_{11}H_{24}$	156
2.53	Dodecane	$C_{12}H_{26}$	170
3.55	Tridecane	$C_{13}H_{28}$	184
4.69	Tetradecane	$C_{14}H_{30}$	198
5.86	Pentadecane	$C_{18}H_{38}$	254
7.03	Hexadecane	$C_{15}H_{32}$	212
8.20	Heptadecane	$C_{17}H_{36}$	240
10.27	Heneicosane	$C_{21}H_{44}$	296
11.27	Eicosane	$C_{20}H_{42}$	282
12.23	Octacosane	$C_{28}H_{58}$	394
13.14	Docosane	$C_{22}H_{46}$	310
14.02	Heptadecane-9-hexyl	$C_{23}H_{48}$	324
14.87	Docosane	$C_{22}H_{46}$	310
15.69	Docosane	$C_{22}H_{46}$	310
16.67	Docosane	$C_{22}H_{46}$	310
17.25	Docosane	$C_{22}H_{46}$	310

Table 3
GCMS data of pure diesel with cationic biocide (DDAB) along with bacterial cultures (ACE 2 and ACE 4)

Retention time	Compound	Molecular formulae	Molecular weight
0.75	*O*-dimethyl benzene	C_8H_{10}	106
1.18	Benzene-(1-methylethyl)	C_9H_{12}	120
1.71	Undecane	$C_{11}H_{24}$	156
2.53	Dodecane	$C_{12}H_{26}$	170
3.55	Tridecane	$C_{13}H_{28}$	184
4.69	Tetradecane	$C_{14}H_{30}$	198
5.40	Dodecane-2,2,4,9,11,11-hexamethyl	$C_{18}H_{38}$	254
5.86	Pentadecane	$C_{15}H_{32}$	212
6.46	Napthalene-1,6,7-trimethyl	$C_{13}H_{14}$	170
7.02	Hexadecane	$C_{16}H_{34}$	226
7.56	Octadecane-3-ethyl-5-[2-ethylbutyl]	$C_{26}H_{54}$	366
8.16	Heptadecane	$C_{17}H_{36}$	240
8.20	Pentadecane-2,6,10,14-tetramethyl	$C_{19}H_{40}$	268
9.24	Octadecane	$C_{18}H_{38}$	254
9.32	Dotriacontane	$C_{32}H_{66}$	450
10.29	Heneicosane	$C_{21}H_{44}$	296
11.28	Eicosane	$C_{20}H_{42}$	282
12.24	Octacosane	$C_{28}H_{58}$	394
13.16	Docosane	$C_{22}H_{46}$	310
14.04	Heptadecane-9-hexyl	$C_{23}H_{48}$	324
15.70	Docosane	$C_{22}H_{46}$	310
16.48	Heptacosane	$C_{27}H_{56}$	380
17.25	Heptacosane	$C_{27}H_{50}$	380
18.17	Octadecane-3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$	366

BRIJ-35) added systems. It reveals that the oxygen addition was taken place in these systems. But in the presence of DDAB, a peak at 1717 cm^{-1} could not be noticed. It can be inferred that the micelle behavior of cationic biocide (DDAB), controls the degradation of diesel.

There is no change in the NMR spectrum of cationic biocide (DDAB), when compared with pure diesel. GC–MS analysis also supports the NMR data. It is well known that cationic biocide like DDAB with a long alkyl chain can form micelles when dissolved in water [35]. In the case of cationic biocide (DDAB) degradation of diesel was not noticed. It can be explained that, cationic biocide (DDAB) has good biocidal activity at the interface. It may be due to the characteristic feature of hydrophilic tail and hydrophobic head of the quaternary ammonium salts. Even though it has micelle formations in water, the positive charge of the biocide attacks the negative charged functional groups in the cell wall of the bacteria. In the case of diesel solution, hydrophilic head enters through the oil–water interface and kills the bacteria (Fig. 5). Hence, degradation of diesel is less when adding quaternary ammonium biocides in diesel phase. But in

Table 4
GCMS data of pure diesel with nonionic biocide (BRIJ-35) along with bacterial cultures (ACE 2 and ACE 4)

Time	Compound	Molecular formulae	Molecular weight
1.71	Undecane	$C_{11}H_{24}$	156
2.53	Dodecane	$C_{12}H_{26}$	170
3.55	Tridecane	$C_{13}H_{28}$	184
4.69	Tetradecane	$C_{14}H_{30}$	198
5.40	2,2,4,9,11,11-Hexamethyl dodecane (*n*-pentadecane)	$C_{15}H_{32}$	212
5.86	Pentadecane	$C_{15}H_{32}$	212
6.46	Napthalene-1,6,7-trimethyl	$C_{13}H_{14}$	170
7.02	Hexadecane	$C_{16}H_{34}$	226
8.16	Heptadecane	$C_{17}H_{36}$	240
10.27	Heneicosane	$C_{21}H_{44}$	296
11.27	Eicosane	$C_{20}H_{42}$	282
12.23	Octacosane	$C_{28}H_{58}$	394
13.14	Docosane	$C_{22}H_{46}$	310
14.02	Heptadecane-9-hexyl	$C_{23}H_{48}$	324
14.87	Docosane	$C_{27}H_{46}$	310
15.68	Docosane	$C_{22}H_{46}$	310
16.48	Docosane	$C_{22}H_{46}$	310
17.25	Docosane	$C_{22}H_{46}$	310

the presence nonionic biocides (BRIJ-35 and TRITON-X-100) kill the microbes only in the diesel phase and fail to kill the microbes present at the oil–water interface (Fig. 6). As we know already microbes are present mainly in water layer which can still carryout the degradation process to larger extent. A new peak can be noticed at 5–6 ppm, which indicates the addition of oxygen during degradation. The NMR spectral study concludes that cationic biocide (DDAB) is better than nonionic biocide (BRIJ-35 and TRITON-X-100). A study of the effects of quaternary ammonium compounds (QACs) on lake microbial communities showed that they had adapted to the toxic effect and became active in biodegradation of the QACs [36].

Table 5
GCMS data of pure diesel with nonionic biocide (TRITON-X-100) along with bacterial cultures (ACE 2 and ACE 4)

Time	Compound	Molecular formulae	Molecular weight
1.71	Tetradecane-1-chloro	$C_{14}H_{29}Cl$	232
2.53	Dodecane	$C_{12}H_{26}$	170
3.53	Tridecane	$C_{13}H_{28}$	184
4.69	Tetradecane	$C_{14}H_{30}$	198
5.86	Penta decane	$C_{15}H_{32}$	212
7.02	Hexadecane	$C_{16}H_{34}$	226
7.56	Octadecane-3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$	366
8.14	Heptadecane	$C_{17}H_{36}$	240
9.23	Octadecane	$C_{18}H_{38}$	254
9.32	Dotriacontane	$C_{32}H_{66}$	450
10.27	Docosane	$C_{27}H_{56}$	380
11.28	Pentacosane	$C_{25}H_{52}$	352
12.23	Octacosane	$C_{28}H_{58}$	394
13.14	Docosane	$C_{22}H_{46}$	310
14.03	Docosane	$C_{22}H_{46}$	310
14.87	Docosane	$C_{22}H_{46}$	310
15.68	Docosane	$C_{22}H_{46}$	310
16.46	Hexacosane	$C_{26}H_{54}$	366
17.25	Nanocosane	$C_{29}H_{60}$	408

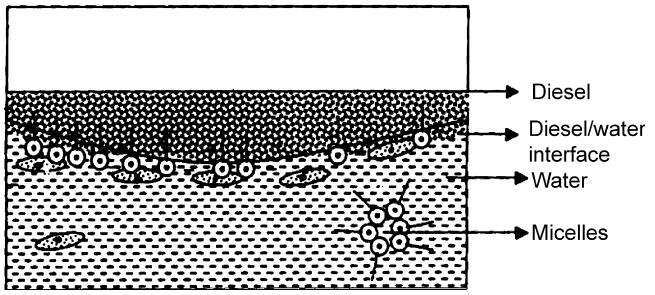

Fig. 5. Cationic biocide (DDAB) in water and oil phase.

The polar head part of cationic biocide (DDAB) tends to remain in the aqueous phase and non-polar tail part tends to hide itself from the water molecules, thus giving raise to micelles. The cationic biocides kill the bacteria by disturbing the arrangements of negatively charged phospholipids in the cell wall while nonionic biocides dissolve the protein present in the cell wall shrink the cell wall and make as lysis condition [37,38]. Basically, a surfactant molecule is made up of two functional groups, a *hydrophilic head group* and a *lipophilic group* [39]. The two groups line up between the oil and water phases with their opposing ends dissolved in the respective phases [40,41]. Besides, the same chemicals have micelle formation characteristic at the water and diesel system [36]. This arrangement creates a third layer at the interface, thus decreasing the interfacial tension between oil and water. When an aqueous phase comes into contact with the oil containing cationic biocide (DDAB) the polar hydrophilic portion of ammonium salt moves towards the biphase/interface and forms an emulsion and thus we will be able to generate a bilayer of cationic biocide (DDAB) which kills the bacteria in the diesel/water interface.

Besides, the results indicate the increasing trend of bacterial growth after fifth day of biocide addition. It can be explained as "regeneration of microbes against biocides". Hence the con-

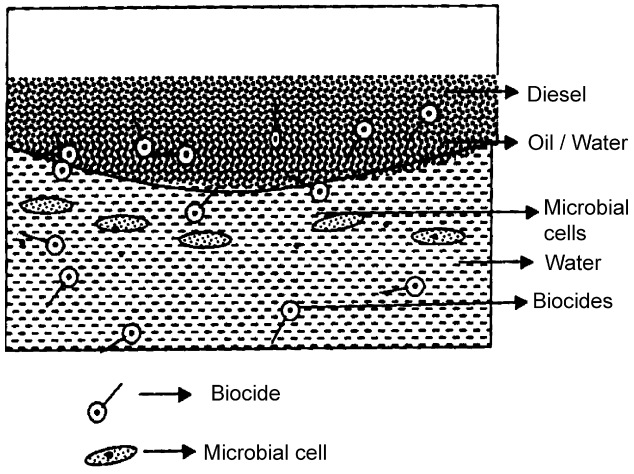

Fig. 6. Nonionic biocides (BRIJ-35 and TRITON-X-100) in water and oil phase.

centration of biocides should be checked continuously, since immunity is also another factor while monitoring the biocides in oil industry. Hence, the present study explains the importance of selection of biocides and monitoring of biocides while applying in oil industry.

5. Conclusions

(1) Micelle formation at the diesel/water interface by DDAB is an important factor for controlling oil degradation.
(2) Cationic biocide (DDAB) is efficient in controlling the oil degradation and shows good biocidal activity at the diesel/water interface when compared to nonionic biocides (BRIJ-35 and TRITON-X-100).
(3) BRIJ-35 and TRITON-X-100 have better bactericidal efficiency than DDAB individual systems of oil medium but it does not work well at the diesel/water interface.

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