SURFACE AND INTERFACE ANALYSIS
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Impact of micelles on the biocidal efficiency in a diesel–water interface

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Biodegradation occurs in the diesel/water interface in petroleum product pipelines. The microbial contamination can result in inhibitor/fuel degradation, leading to unacceptable levels of turbidity, filter plugging, storage tank corrosion and stored product souring. Therefore, selection of the biocide/inhibitor plays an important role in the transportation of petroleum products through pipelines. Three biocides (cationic and nonionic) were employed to study the biodegradation in a diesel-water interface. The biocidal efficiency against degradation of diesel was examined by employing Fourier-transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and gas chromatography mass spectrometry (GC-MS) techniques. Bronopol (2-bromo-2-nitro-propane-1, 3-diol) was found to have higher bactericidal efficiency than *N*-cetyl-*N,N, N*-trimethyl ammonium bromide (CTAB) and cetyl pyridinum bromide (CPB). But the cationic biocides (CTAB and CPB) showed good biocidal efficiency at the interface. The data are explained in terms of a model that postulates the formation of a 'micelle' at the diesel-water interface.

KEYWORDS: biocide; micelle; interface; surfactants

INTRODUCTION

Microorganisms have the ability to utilize hydrocarbons as their sole source of carbon and are widely distributed in nature. Microbiologically influenced corrosion (MIC) in oil pipelines is mainly associated with different types of bacteria and fungi.[1–7] MIC is responsible for the leakage in steel tanks, souring of fuels and failure of pipelines. A comprehensive US Environmental Protection Agency report documents that 6–10% of tank failures are caused by internal corrosion. Therefore, attention has been turned towards the control of microorganisms.[8–11] Hence, it is important to have knowledge of microbial problems occurring in storage tanks and pipelines to develop methods or propose designs, so as to minimize oil degradation/pipeline failures.[12,13] Jana *et al*.[14] noticed a failure in an oil pipeline at the Mumbai offshore. They suggested that the combined effect of carbon dioxide (CO_2), sulfate reducing bacteria (SRB) and chloride in the low velocity area caused the severe corrosion and failure of the 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] A number of surfactants have been isolated from microbial cultures following the growth of bacteria and fungi on a variety of aliphatic hydrocarbons. 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 utilization of hydrocarbons and this, in turn, leads to the growth of microbial cells, which has important implications for the oil industry.[25] In order to control the effects of microbial growth, several lines of approach have been used, e.g. good house keeping practices, treatment with biocides to limit their growth, 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 the emulsion[25] and diesel degradation. Therefore, the identification of inhibitors/biocides that could act at the interface is needed in pipelines transporting petroleum. Since the characteristics of biocides are not evaluated properly before use, many misapplications of biocides occur resulting in MIC.[12,27] In the present investigation, two cationic compounds and one nonionic compound have been selected to control the bacterial degradation process. A mechanism of the biocidal action is proposed along with solution chemistry.

EXPERIMENTAL

Sample collections and bacteria used

The bacterial strains *Serratia marcescens* ACE2 and *Bacillus cereus* ACE 4[28] used in this study were isolated from an oil-transporting pipeline of oil refineries in north-west India (The nucleotide sequences data has been deposited in GenBank under the sequence numbers DQ092416 and AY912105.) In

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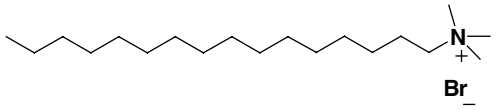
the present study, the two bacteria, namely, *B. cereus* and *S. marcescens*, were selected and the micelle behavior of the biocidal effect on microbes in petroleum products was investigated.

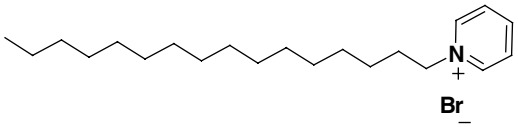
Biocides employed

Three biocides (two cationic and one nonionic) were employed to study their efficacy towards hydrocarbon utilizers.

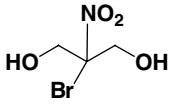
(1) CTAB – *N*-cetyl-*N*, *N*, *N*-trimethyl ammonium bromide



(2) CPB – cetyl pyridinum bromide



(3) BNP – 2-bromo-2-nitro-propane-1,3-diol (bronopol)



Preparation of the water solution of biocides

The respective biocides (0.1 g) were dissolved in 100 ml of distilled water in separate makeup flasks to give a concentration of 1000 ppm and this was taken as the stock solution (SS). Ten milliliters of the SS was added to 200 ml of Bushnell–Hass (BH) broth, which gave a concentration of 50 ppm of water-soluble (WS) biocides.

Preparation of the diesel solution of biocides

The respective biocide (0.1 g) was dissolved in 100 ml of the solvent (ethylene glycol monobutyl ether) in a makeup flask to give a concentration of 1000 ppm and this was taken as the SS. Ten milliliters of the SS was added to 200 ml of BH broth, which gave a concentration of 50 ppm of diesel-soluble (DS) biocides.

Biodegradation study

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

Analytical methods

At the end of 30 days of incubation, the residual diesel was extracted with an equal volume of dichloromethane. Evaporation of the solvent was carried out in a water bath at 40°C. The resulting solution (1 µl) was analyzed using Fourier-transform infrared (FTIR), NMR and gas chromatography mass spectrometry (GC-MS). FTIR (Perkin Elmer, model paragon 500) was used to detect the functional groups of the compounds. 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 crystals and made into pellets, which were fixed in the sample holder, and the spectrum was recorded in the transmittance mode. ^1H NMR (Bruker 200 MHz) was used to detect the protons of the nuclei in the compound. Deuterated chloroform was used as the solvent. Tetramethylsilane (TMS) was used as the internal reference standard. Twenty microliters of the sample was taken for the analysis. The biodegradation of diesel was monitored quantitatively by GC-MS analysis, as described by Luigi Michaud *et al*.[29] One microliter of the resultant corrosion inhibitor solution was analyzed by a Thermo Finnigan GC-MS instrument (Trace MS equipped with an RTX-5 capillary column (30 m long ×0.25 mm i.d.)) with high-purity nitrogen as the carrier gas. The oven was programed between 80 and 250 °C at a rate of heating of 10°C/min. The GC retention data of the inhibitor correspond to the structural assignments carried out after an national institute of standards and technology (NIST) library search with a database and mass spectral interpretation. The degradation of diesel as a whole was expressed as the percentage of diesel degraded in relation to the amount of the remaining fractions in the appropriate abiotic control samples (pure diesel). The biodegradation efficiency (BE), based on the decrease in the total concentration of diesel hydrocarbons, was evaluated by using the following expression:

$$BE(\%) = 100 - (A_s \times 100/A_{ac})$$

where A_s is the total area of the peaks in each sample, A_{ac} is the total area of the peaks in the appropriate abiotic control and $BE(\%)$ is the efficiency of biodegradation.

RESULTS AND DISCUSSIONS

Microbial contamination of fuels has been the cause of intermittent operational problems throughout the world for

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many years, and more recently the frequency and severity of the cases appear to be increasing dramatically.[5] Jobson *et al*.[30] also reported that intermediate hydrocarbon degradation products serve as energy sources for the physiological activities of the SRB *Desulfovibrio* sp., and explained as the reason for the intense MIC in the Pembiana oil pipeline. Samant and Anto[31] reported SRB in oil pipelines and noticed the interaction between chloride ions and SRB on corrosion. A strain of the SRB *Desulfovibrio* sp. was isolated from the microbial communities involved in MIC in a gas- and oil-transporting pipeline in the Gulf of Mexico by Janet Jan[32] and Mora- Mendoze.[33] Petroleum pipeline industries add preservatives/biocides to avoid the contamination or degradation of petroleum products. The major problem associated with such contamination is the formation of microbial biomass at the oil–water interface. Emulsifying agents are produced by the microbial cells to aid in the uptake of the hydrocarbon prior to metabolism, when the emulsion contains a large number of microorganisms.[34,35] The selection of the biocide is based on its mechanism of action at the water/oil interface, which is an important factor while procuring biocides from the market. Since pipeline industries are facing some problems in the selection of inhibitors/biocides, the present study was undertaken to investigate the behavior of two cationic biocides, CTAB and CPB, and one nonionic biocide, BNP, on diesel degradation. In the present study, the role of micelle formation on the killing effect of bacteria was investigated.

Enumeration of bacteria during degradation

The bacterial count of the BH broth supplemented with diesel (without biocide addition as control) was monitored at regular intervals of 5 days, and those of the test samples (with a biocide concentration of 50 pm) were also recorded in comparison with control, and the results are tabulated in Table 1. It is evident that the bacterial isolates were able to utilize/degrade the diesel sample, which can be concluded with the corresponding increase in the bacterial population. A low recovery of the bacterial population was recorded at the 5th day of incubation in all DS biocides. An exponential growth was observed in the control (without biocide addition). In the WS system, the test sample (with biocides) shows initially a lower bacterial count when compared with the control, which is about 4.7×10^4 colony forming unit/mL (CFU/mL). After 15 days of incubation, a gradual increase in the growth was observed, and on the 30th day, the control showed too numerous to count (TNTC), while significant decreases the count was noticed in all the biocide systems.

Table 1. pH values of the BH broth during degradation

		pH values Incubation period (days)						
System	Layer	Initial	5	10	15	20	25	30
Control	–	7.27	7.33	7.37	7.48	7.53	7.65	7.75
CTAB	WS	7.25	7.3	7.31	7.33	7.34	7.37	7.48
	DS	7.35	7.34	7.34	7.36	7.38	7.40	7.42
CPB	WS	7.26	7.28	7.30	7.33	7.38	7.42	7.46
	DS	7.36	7.26	7.29	7.29	7.32	7.37	7.43
BNP	WS	7.42	7.37	7.26	7.15	7.07	7.07	6.86
	DS	7.36	7.30	7.25	7.27	7.30	7.21	7.36

pH values of the BH broth: 7.0 ± 0.2.
WS, water-soluble biocide; DS, diesel-soluble biocide; Control, without biocide.

Table 2. Enumeration of bacterial count during degradation in the presence/absence of biocides

		Total viable count (CFU/ml) Incubation period (days)					
System	Layer	5	10	15	20	25	30
CTAB	WS	3.6×10^3	4.3×10^4	5.7×10^5	9.8×10^6	1.27×10^8	TNTC
	DS	TLTC	1.5×10^3	2.8×10^4	7.7×10^6	4.2×10^7	9.7×10^8
CPB	WS	2.4×10^3	5.2×10^4	6.7×10^5	7.4×10^6	1.3×10^8	TNTC
	DS	TLTC	3.4×10^3	7.3×10^4	9.7×10^6	3.7×10^7	TNTC
BNP	WS	1.7×10^3	3.7×10^4	5.2×10^4	7.3×10^6	3.7×10^8	TNTC
	DS	TLTC	TLTC	3.6×10^4	9.3×10^5	1.6×10^6	1.8×10^7
Control	–	4.7×10^4	5.6×10^5	9.7×10^7	TNTC	TNTC	TNTC

WS, water-soluble biocide; DS, diesel-soluble biocide; TNTC, too numerous to count; TLTC, too low to count; control, without biocide.

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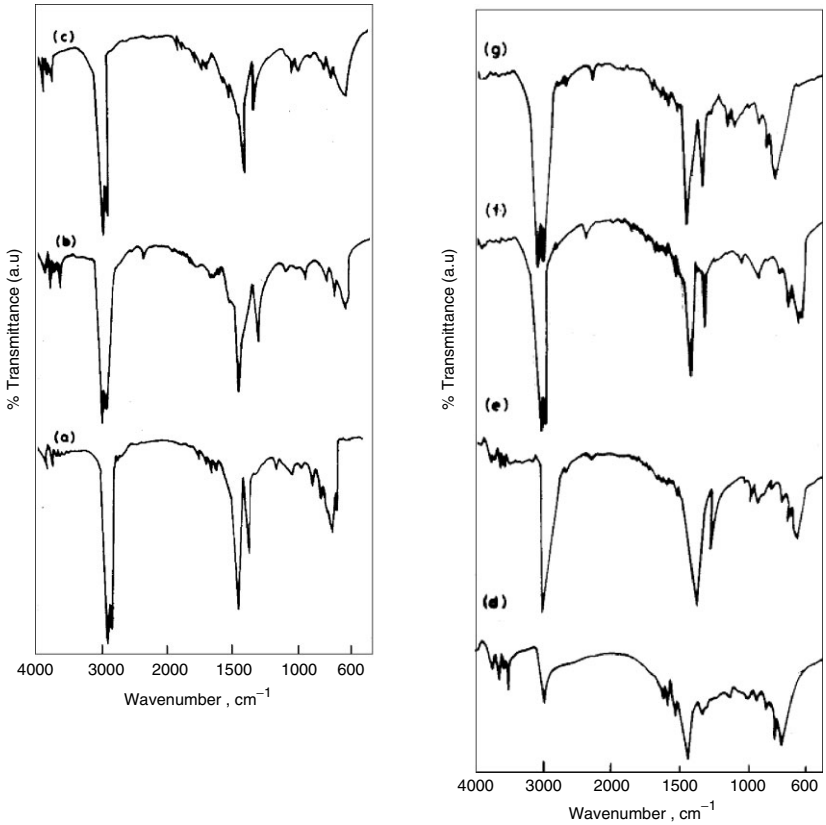

Figure 1. FTIR spectrum of (a) pure diesel, (b) CTAB – water soluble, (c) CTAB – diesel soluble, (d) CPB – water soluble, (e) CPB – diesel soluble (f) BNP – water soluble, (g) BNP – diesel soluble.

pH measurements

The pHs of the test samples and control were monitored regularly at intervals of 5 days, and the results are tabulated in Table 2. The pH of the BH broth was 7.0 ± 0.2. The initial pH of the test samples was between 7.26 and 7.42 on the first day of incubation. On the 30th day of incubation, the pH of the control sample was 7.75 and for the BNP-added system the lowest pH value was 6.86. No significant difference in pH could be noticed.

FTIR analysis of diesel degradation

The IR spectrum of pure diesel (Fig. 1(a)) shows the characteristic bands at 2919, 2854 (C–H aliphatic stretch); 1744 (C=O carbonyl group); 1649 (C=C conjugated diene); 1607 (C=C stretch in aromatic nuclei); 875, 808 (C–H deformation for disubstituted benzene) and 724 cm^{-1} (monosubstituted benzene). The IR spectrum of WS CTAB shows (Fig. 1(b)) the characteristic bands at 2920, 2854 (C–H aliphatic stretch); 1743 (C=C carbonyl group); 1693 (C=C conjugated diene); 1539, 1507 (C=C stretch in aromatic nuclei), 1458, 1375 (CH deformation of the methyl group); 724 (monosubstituted benzene) and 873, 809 cm^{-1} (meta-disubstituted benzene). The IR spectrum of DS CTAB shows (Fig. 1(c)) the characteristics bands at 2920, 2854 (C–H aliphatic stretch); 1744 (C=O carbonyl group); 1648 (C=C conjugated diene); 1458, 1375 (CH deformation of the methyl group); 876, 810 (meta-disubstituted benzene) and 724 cm^{-1} (monosubstituted benzene). The IR spectrum of WS CPB shows (Fig. 1(d)) the characteristics bands at 2920, 2853, 2728 (C–H aliphatic stretch); 1744, 1698 (C=O carbonyl group); 1649 (C=C conjugated diene); 1608, 1539 (C=C stretch in aromatic nuclei); 1458, 1375 (CH deformation of the methyl group); 875, 809 (meta-disubstituted benzene) and 743, 723 cm^{-1} (mono substituted benzene). The IR spectrum of DS CPB shows (Fig. 1(e)) the characteristic bands at 2926, 2855, 2728 (C-H aliphatic stretch); 1744, 1682 (C=C carbonyl group); 1649 (C=C conjugated diene); 1608 (C=C stretch in aromatic nuclei); 1458, 1375 (CH deformation of the methyl group); 875, 809 (meta-disubstituted benzene) and 742, 723 cm^{-1} (monosubstituted benzene). The IR spectrum of WS BNP shows (Fig. 1(f)) the characteristic bands at 2926, 2855, 2728, 2360 (C–H aliphatic stretch); 1744, 1681 (C=C stretch in aromatic nuclei); 1607, 1539 (C=C stretch in aromatic nuclei); 873, 809 (C–H deformation of disubstituted benzene) and 742, 723 cm^{-1} (monosubstituted benzene). The IR spectrum of DS BNP shows (Fig. 1(g)) characteristic bands at 2920, 2854, 2728 (C–H aliphatic stretch); 1744, 16 781 (C=O carbonyl group); 1649 (C=C conjugated diene); 1606, 1539 (C=C stretch in aromatic nuclei); 1458, 1375 (CH deformation of the methyl group); 876, 809 (meta-disubstituted benzene) and 742, 723 cm^{-1} (monosubstituted benzene).

NMR analysis of diesel degradation

The spectrum of pure diesel shows (Fig. 2) peaks at 0–3 ppm, which indicates the presence of aliphatic protons. Another peak at 6–7 ppm indicates the presence of aromatic protons. The NMR spectrum obtained after treatment with the water and diesel solutions of CTAB shows (Fig. 2) the peaks at 0–3 ppm, which indicates the presence of aliphatic

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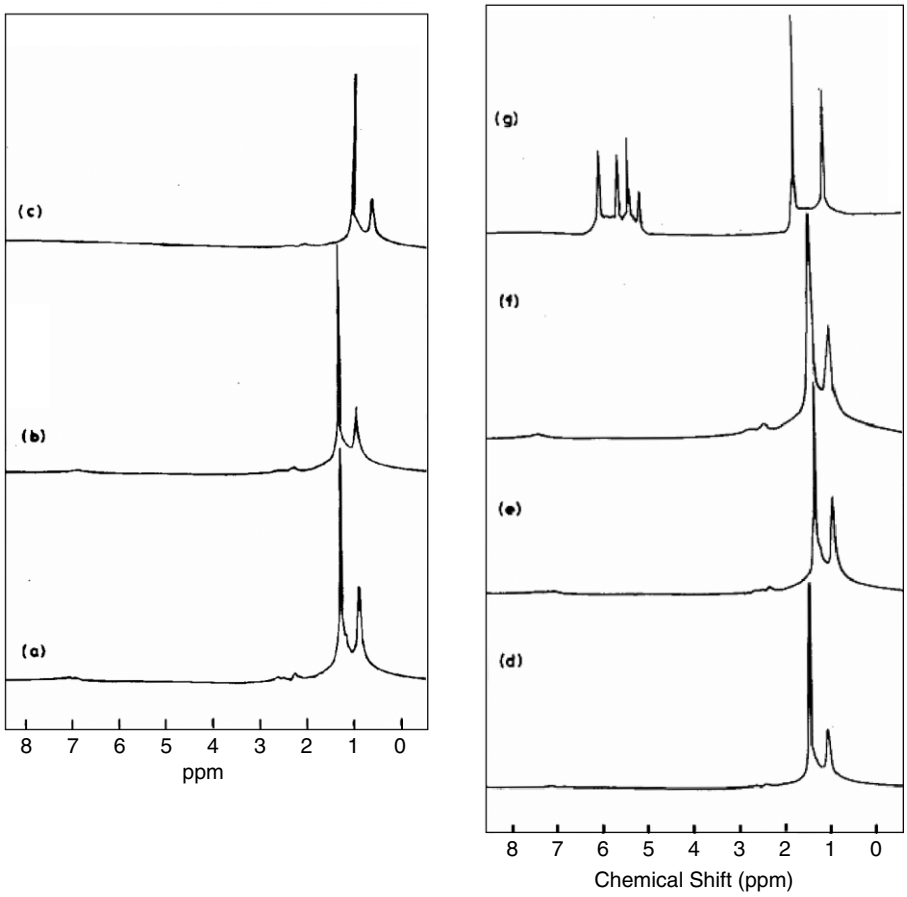

Figure 2. The NMR spectrum of (a) pure diesel, (b) CTAB – water soluble, (c) CTAB – diesel soluble, (d) CPB – water soluble, (e) CPB – diesel soluble (f) BNP – water soluble, (g) BNP – diesel soluble.

protons. Another peak at 6–7 ppm indicates the presence of aromatic protons. Similarly, water solutions of CPB and BNP as well as the diesel solution of CPB showed the same result. However, the NMR spectrum of diesel obtained after treatment with a diesel solution of BNP showed additional peaks at 5–6.5 ppm, in addition to peaks at 0–3 and 6–7 ppm. This indicates the formation of oxygen-included protons $(O–CH_2)_n$, and this may be due to the bacterial metabolism. Therefore, degradation of diesel has been noticed in DS systems. It is concluded that the use of either biocide in the WS or DS form minimized the degradation to a large extent.

The IR spectrum of diesel shows peaks at 1744, 1681 (C═O carbonyl group) and 1649 cm^{-1} (C═C conjugated diene), which indicate the presence of a carbonyl group and C═C conjugated diene. The peaks at 3745 and 3837 cm^{-1} indicate the presence of N–H stretch. Generally, amine-based carboxylic acid is used as a corrosion inhibitor in petroleum product pipelines. Hence, the presence of the carboxyl group may be due to the addition of corrosion inhibitors in the product sample. However, in the control system (without biocide) after the degradation study the above peaks were not noticed. This can be explained as due to the absence of inhibitory compounds containing the above functional groups (N–H (amine) and C═O (carbonyl)), which were consumed by the microbes.

In the FTIR spectrum of diesel samples obtained after treatment with the diesel or water solution of biocides, the presence of N–H stretch (3744, 3614 cm^{-1}), C–H aliphatic stretch (2920, 2854 cm^{-1}), CO–NH stretch (1829, 1867 cm^{-1}), CH deformation of the CH_3 group (1458, 1375 cm^{-1}) and substituted benzene peaks (873, 809, 724 cm^{-1}) were seen. The presence of (CO–NH) amide in diesel may be due to the addition of biocides in both water and solvent systems.

There is no change in the NMR spectrum of CTAB and CPB when compared to that of pure diesel. From the results it is evident that DS biocides are more efficient than WS biocides. It is well known that quaternary ammonium salts (QASs), such as CTAB and CPB, with a long alkyl chain can form micelles when dissolved in water.[36] In the case of CTAB and CPB, degradation of diesel was not noticed. It can be explained by the fact that CTAB and CPB have good biocidal activity at the interface. It may be due to the characteristic features of the hydrophilic tail and the hydrophobic head of QASs. Even though it shows micelle formation in water, the positive charge of the biocide attacks the negatively charged functional groups in the cell wall of the bacteria. In the case of diesel solution, the hydrophilic head enters through the oil–water interface and kills the bacteria (Fig. 3). Hence, degradation of diesel is less when adding QAS biocides in the diesel phase.

GC-MS analysis of diesel degradation

The GC retention data of the diesel corresponding to structural assignments carried out after an NIST library

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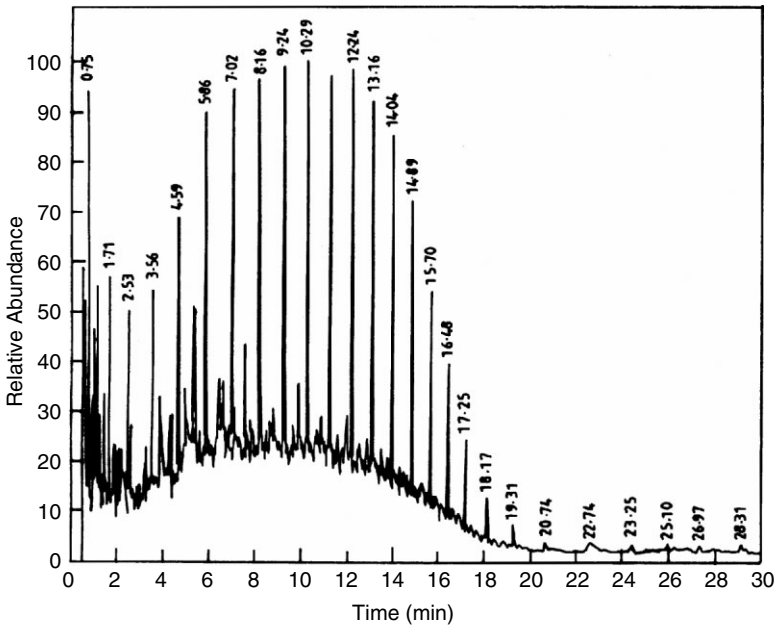

Figure 3. GC-MS spectrum of pure diesel.

Table 3. Biodegradation efficiency data of pure diesel with various biocides (CTAB, CPB and BNP)

		Pure diesel	CTAB		CPB		BNP	
RT (min)	Compound	RA	RA	BE (%)	RA	BE (%)	RA	BE (%)
2.53	Dodecane ($C_{12}H_{26}$)	51	50	1.97	50	1.97	51	0
3.55	Tridecane ($C_{13}H_{28}$)	65	65	0	60	7.7	59	9.24
4.69	Tetradecane ($C_{14}H_{30}$)	69	69	0	69	0	68	1.45
5.40	2,2,4,9,11,11-Hexamethyldodecane ($C_{15}H_{32}$)	91	91	0	90	1.1	90	1.1
7.02	Hexadecane ($C_{16}H_{34}$)	95	95	0	95	0	95	0
8.16	Heptadecane ($C_{17}H_{36}$)	98	98	0	95	3.07	92	6.13
9.24	Octadecane ($C_{18}H_{36}$)	99	98	1.02	92	7.08	92	7.08
10.29	Heneicosane ($C_{21}H_{44}$)	100	99	1.00	98	2.00	96	4.00
11.28	Eicosane ($C_{20}H_{42}$)	98	98	0	98	0	92	6.23
12.24	Octacosane (C_{28} H_{58})	98	98	0	89	10.21	88	10.21
13.16	Docasane ($C_{22}H_{46}$)	95	95	0	80	12.64	80	15.79
14.04	Heptadecane, 9 hexyl	85	85	0	68	16.48	65	23.53
14.89	Tricosane ($C_{23}H_{48}$)	73	73	0	55	13.70	56	23.29
15.70	Docosane ($C_{27}H_{56}$)	65	65	0	39	30.77	38	41.54
16.48	Heptacosane ($C_{27}H_{56}$)	40	40	0	28	17.50	21	47.50
17.25	Heptacosane ($C_{27}H_{50}$)	25	24	4.00	12	12.00	11	56.00
18.17	Octadecane 3-ethyl-5-(2-ehtyl butyl)	13	13	0	8	15.39	9	30.71
	BE(total percentage)			0.47		8.91		16.69

RT, retention time; RA, relative abundance; BE, biocidal efficiency.

search with a database and by mass spectral interpretation are presented in Table 3. From the GC-MS spectral analysis (Fig. 3), it is observed that the diesel (uninoculated system) consists of aliphatic and aromatic hydrocarbons, including *O*-dimethyl benzene, 1-methylethyl-benzene, undecane ($C_{11}H_{24}$), dodecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$), tetradecane ($C_{14}H_{30}$), 2,2,4,9,11,11-hexamethyl dodecane (*n*-penta decane, $C_{15}H_{32}$), pentadecane ($C_{15}H_{32}$), 1,6,7-trimethylnapthalene ($C_{13}H_{14}$), hexadecane ($C_{16}H_{34}$), octadecane (3-methyl-15-C_2 ethylbutyl), heptadecane ($C_{17}H_{36}$) (240), pentadecane 2,6,10,14-tetramethyl, octadecane ($C_{18}H_{36}$) (*n*-octadecane), dotriacontane ($C_{32}H_{66}$) (*n*-dotriadecane), henricosane ($C_{21}H_{44}$) eicosane ($C_{20}H_{42}$), octacosane ($C_{28}H_{58}$), docasane ($C_{22}H_{46}$) heptadecane, 9-hexyl, tricosane ($C_{23}H_{48}$) docosane ($C_{27}H_{56}$) heptacosane ($C_{27}H_{56}$) heptacosane ($C_{27}H_{50}$) cotadecone 3-ethyl-5-(2-ehtyl butyl) and dotria contane ($C_{32}H_{66}$). These compounds are the major components present in the diesel (without the bacterial system).

The CTAB and CPB inoculated systems show (Figs 4 and 5) the aliphatic and aromatic components, which are

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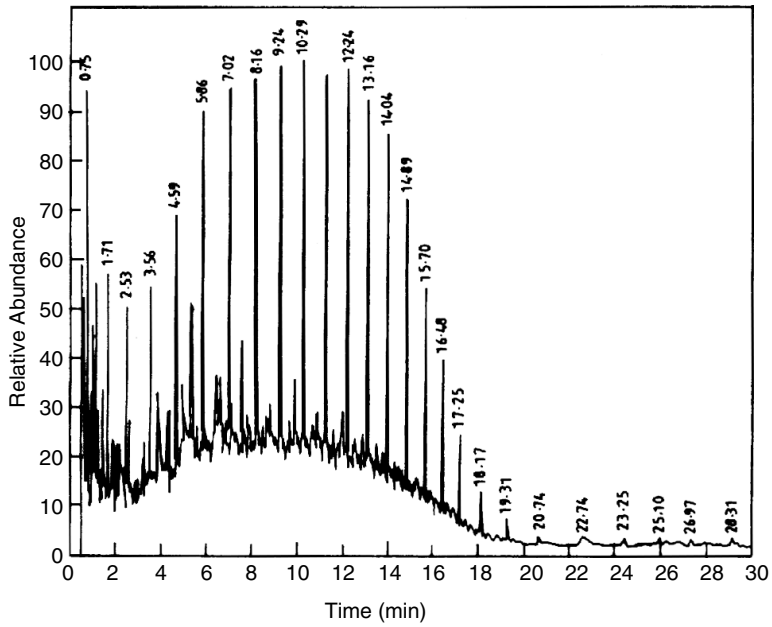

Figure 4. GC-MS spectrum of diesel with cationic biocide CTAB with mixed cultures.

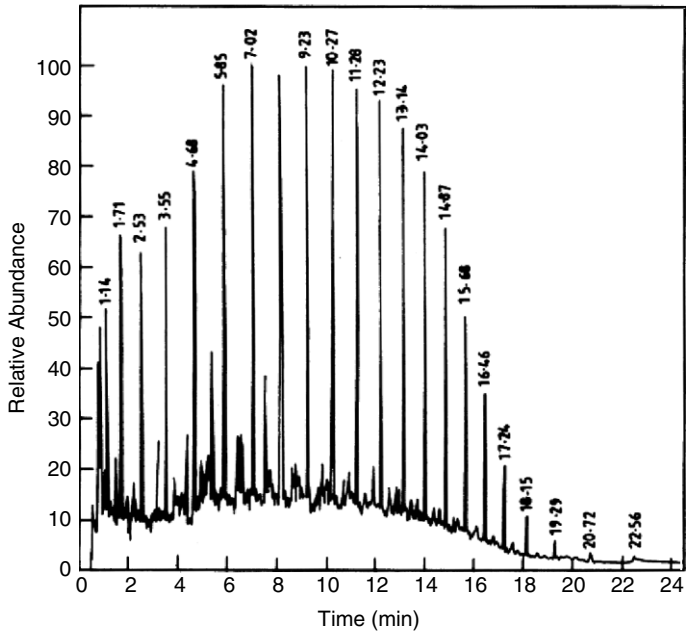

Figure 5. GC-MS spectrum of diesel with cationic biocide CPB with mixed cultures.

similar to that of the control system (without bacteria). There is no new compound formation in the presence of cationic biocides (CTAB and CPB). But in the presence of nonionic biocides (BNP) (Fig. 6), a new peak can be observed at 1.14 min retention time, which indicates the presence of benzene (1-methylethyl). Also, a peak reduction is noticed at 13.16, 14.04, 14.89, 15.70, 16.48, 17.25, 18.17, 19.31 min retention time, which indicates docasane, heptadecane, 9-hexyl, tricosane, docosane, heptacosane, heptacosane, octadecane 3-ethyl-5-(2-ehtyl butyl) and dotria contane, respectively. It reveals that the bacteria consume small quantities of these compounds. This is due to the presence of nonionic biocide, which could not kill the microbes at the oil–water interface because BNP does not have the micelle character. The BE of pure diesel by the strains ACE2 and ACE4 are presented in the Table 3. BNP inoculated with the diesel system showed a maximum degradation efficiency of 16.69%. But in the CTAB and CPB inoculated systems, the degradation efficiency is only 0.47% and 8.91%, respectively. It shows that the cationic compound stops diesel degradation.

Because of the WS nature of BNP, it disperses throughout the surface and kills the microbes in the water phase and reduces the bacterial attack at the water–diesel interface (Fig. 7). But, the DS biocide BNP kills the microbes only in the diesel phase and fails to kill the microbes present at the oil–water interface. As we know already, microbes are present mainly in the water layer, which can still carryout

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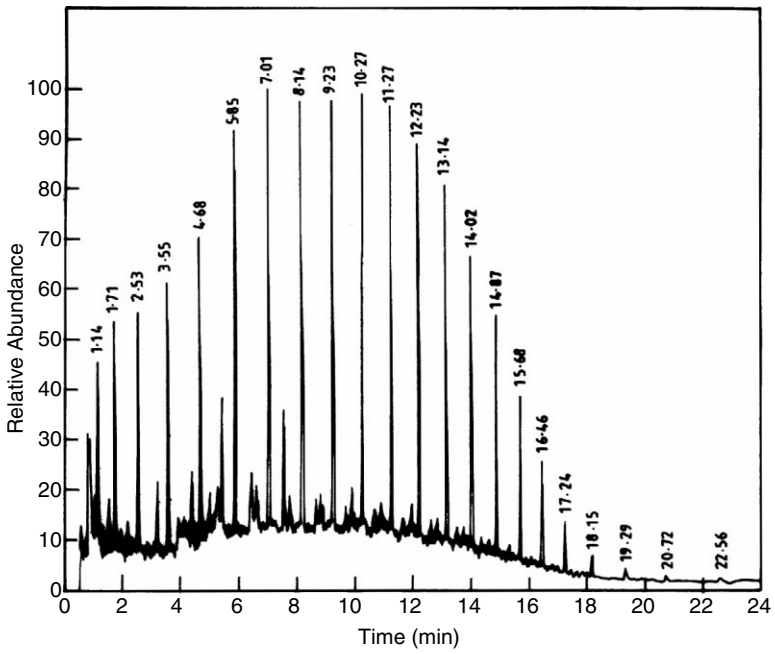

Figure 6. GC-MS spectrum of diesel with the nonionic biocide bronopol with mixed cultures.

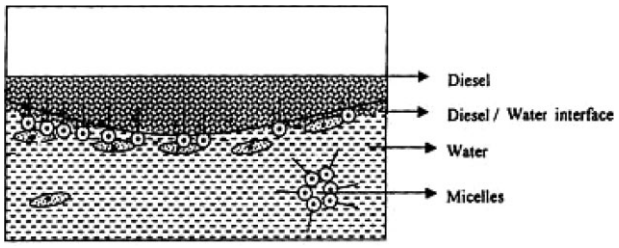

Figure 7. Nonionic biocides in water and oil phases (BNP).

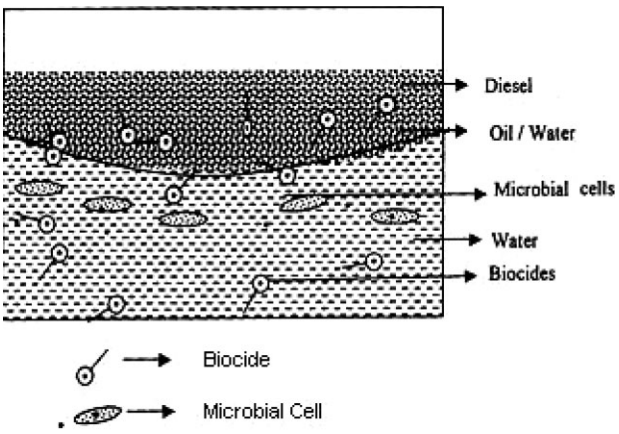

Figure 8. Cationic biocides in water and oil phases (CTAB and CPB).

the degradation process to a 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 CTAB and CPB are better than BNP. A study of the effects of QASs on the microbial communities in lakes showed that the microbial communities have adapted to the toxic effect and become active in biodegradation of the QASs.[37] The resistance of *Pseudomonas aeruginosa* to QASs has been reported in another study. For *Acinetobacter calcoaceticus*, it was demonstrated that increased resistance to QASs allowed the isolation of mutants with enhanced capsule production.

The polar head of CTAB/CPB tends to remain in the aqueous phase and the nonpolar tail tends to hide itself from the water molecules, thereby giving raise to micelles (Fig. 8). The toxic effect of surfactants on bacteria can be explained by two main factors:[38] (i) disruption of cellular membranes by interaction with lipid components; and (ii) reactions of surfactant molecules with proteins essential to the functioning of the cell. 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 a lysis condition.[39] At pH 7 and above, cationic surfactants are the most toxic ones, while anionic surfactants display the most toxic behavior at lower pH values. Nonionic surfactants are in general less active against bacteria than ionic ones. Basically, a surfactant molecule is made up of two functional groups, a hydrophilic head group and a lipophilic group.[40] These two groups line up between the oil and water phases with their opposing ends dissolved in the respective phases.[41] Besides, the same chemicals have micelle-formation characteristics in the water and diesel systems.[37] This arrangement creates a third layer at the interface, thereby decreasing the interfacial tension between oil and water. When an aqueous phase comes into contact with the oil containing CTAB/CPB, the polar hydrophilic portion of the ammonium salt moves toward the biphase/interface and forms an emulsion, and therefore we will be able to generate a bilayer of CTAB/CPB.

When a bacterial species wants to use hydrocarbon for its survival, it has to cross the bilayer formed by the CTAB/CPB and could be killed easily or will get affected. In the case of CTAB/CPB dissolved in water, the formation of an emulsion is not possible because they get localized inside the water in the form of micelles. Thus, when CTAB/CPB in dissolved

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in oil, it will be more effective when compared to biocides dissolved in water. BNP was also used as a biocide by dissolving it both in the water and oil phases; when it is dissolved in the oil phase, the compounds disperse freely in the oil and their polar heads tend to be out of the oil phase, the whole oil system becomes poisonous for microbial growth and therefore the biocide in the oil phase significantly kills the bacteria when compared with when dissolved in water.

Besides, the results indicate the increasing trend of bacterial growth after the 5th day of biocide addition. It can be explained as 'regeneration of microbes against biocides'. Hence the concentration of biocides should be checked continuously, since immunity is also another factor while monitoring the biocides in the oil industry. Hence, the present study explains the importance of the selection and monitoring of biocides while applying to the oil industry.

CONCLUSIONS

(1) Micelle formation at the diesel/water interface by CTAB and CPB is an important factor for controlling oil degradation.
(2) CTAB and CPB are efficient in controlling oil degradation and show good biocidal activity at the diesel/water interface.
(3) BNP has better bactericidal efficiency than CTAB and CPB individual systems of oil and water media because it does not have micelle-forming property.

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