

Biocide Action on Bacterial Induced Corrosion

Mataqi K*, Jose S and Mathew B

Kuwait Institute for Scientific Research, Kuwait.

***Correspondence:**Khalil Mataqi, Kuwait Institute for Scientific Research, Kuwait,
E-mail: kmataqi@hotmail.com.**Received:** 10 January 2018; **Accepted:** 19 February 2018**Citation:** Mataqi K, Jose S, Mathew B. Biocide Action on Bacterial Induced Corrosion. *Microbiol Infect Dis*. 2018; 2(1): 1-7.**ABSTRACT**

The presence and growth of bacteria in the oil industry especially in the fuel storage tanks results in costly maintenance measure as well as alteration in oil product specifications. The aim of this study is to screen the effect of the biocide (Predator 6000) on controlling the bacterial growth in the oil storage tanks in Kuwait, to identify the optimum dosage of the biocide needed to control bacterial growth in petroleum product, and to analyze the fuel quality after treatment with the biocide. Minimum inhibitory concentration of the Predator 6000 were estimated and used for detailed study. Predator 6000 was examined for its chemical composition, minimal bacterial concentration (MBC) and the coupon weight loss study. The biocide showed an effectiveness in the prevention of bacterial growth; did not change weight of mild steel coupons (custom made circular coupons of an average 2.45 cm diameter and 0.35 cm thickness, which were made from the same material as of the fuel storage tank were used to check whether the biocide have a corrosive effect); and did not affect the quality of fuels. Furthermore, under the electron microscopy study, it was proved that Predator 6000 treated coupons had a negligible degree of corrosion.

Keywords

Bacteria, Biocide, Predator 6000, Electron microscopy.

Introduction

Biocide is defined as a chemical substance or composition used to kill microorganisms and/ pest organisms considered to be undesirable [1]. Biocides, which are oxidizing or non-oxidizing, are used widely to control microbial induced corrosion (MIC). Chlorine, bromine, and ozone are typical oxidizing biocides, while non-oxidizing biocides include formaldehyde, glutaraldehyde, isothiazolones, and quaternary ammonia compounds [2]. Oxidizing biocides are effective only in freshwater because they are inactivated by organic matter. Non-oxidizing biocides are more effective as they can control bacteria, fungi and algae and are pH independent. Combinations of oxidizing and non-oxidizing biocides are also used. Laboratory studies proved that isothiazolone mixture and quaternary ammonium compounds are more effective than glutaraldehyde and formaldehyde releasing agents [3]. But toxicity of biocides leads to environmental concerns.

Biocides act by interacting with external cellular structures (e.g. Ethylenediaminetetraacetic acid, glutaraldehyde, phenols etc.), cell membranes (ethanol, surfactants, i-propanol, etc.), and cytoplasm (alkylating agents, proflavine, aldehydes, arylmethan,

and acridine dyes). Requirements of good biocides include high biocidal activity and wide spectrum of action; long-term action; good solubility in water and hydrocarbons; effective activity at low concentration without fuel deterioration; chemical and thermal stability; corrosion-inertness with regard to the equipment used; ecological safety; compatibility with components of borehole solutions without changing their physical and chemical properties; not causing damage of catalysts at oil refining factories; made from cheap and accessible raw materials; and cost effectiveness [4-6].

4-(2-nitrobutyl) morpholine and 4, 4'-(2-ethyl-2-nitrotrimethylene)-dimorpholine Blend: These morpholine derivatives comes under C- hydroxymethyl compounds (Figure 1). Monomethylol nitropropane and dimethylol intermediates react with morpholine to form the end products [7]. This mixture is highly soluble in nonpolar solvents. A concentration between 500 and 1000 ppm is active against microbial deterioration. The blend is active against bacteria and fungus. Even though they are produced from formaldehyde, morpholine, and nitropropane, they do not exert their antimicrobial effect through the release of formaldehyde [8].

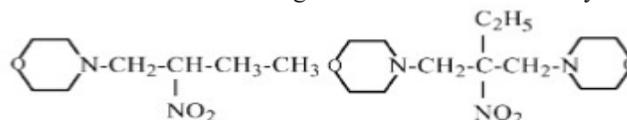


Figure 1: Structure of 4-(2-nitrobutyl) morpholine and 4, 4'-(2-ethyl-2-nitrotrimethylene)-dimorpholine [7].

Materials and Methods

The present study aimed to monitor and control bacterial growth and activity in the fuel storage tanks in Kuwait. The biocide, Predator 6000 (Innospec Limited, UK) was imported for evaluation of its inhibitory effect on bacteria causing corrosion in fuel storage tanks. A group of four microbial strains (*Bacillus sp.*, *Brachy bacterium sp.*, *Kocuria sp.*, and *Staphylococcus sp.*), which are most active, the principal groups isolated from different Kuwait storage fuel tanks [9], and are able to cause pitting, local spotting (start of corrosion), and biofilm formation were used as the microbial source.

In fuel storage tanks, all bacterial species coexist, and to reproduce this situation, a mix culture (prepared by mixing the four selected bacterial cultures) was also used. The inhibitory effects of the biocide were investigated by using agar well diffusion method.

Screening of the Biocide by Agar Well Diffusion Method and Estimation of Minimum Inhibitory Concentration

Nine concentrations from the biocide ranging from 100 ppm to 32,000 ppm were tested. Overnight, nutrient broth cultures of the four bacterial strains and the mix cultures were spread uniformly on to nutrient agar plates. Biocide (150 µl) was placed in wells within the nutrient agar plates. Control plates (without biocide) for each bacterial strain and mix culture were also maintained. Triplicate plates were kept for each concentration.

The inhibitory effect was measured by the size of the inhibitory zone formed around the well. Zone diameters were measured after incubating at 37°C for 24 hrs. Minimum inhibitory concentration was determined as the antilog of the zero intercept of a linear regression of the squared size of these inhibition zone radii (in cm) plotted against the logarithm of the biocide concentration [10].

Estimation of Minimum Bactericidal Concentration

Minimum bactericidal concentration (MBC) is the minimal amount of antibiotic that results in a ≥99.9% decrease in the initial inoculum within 24 hrs in a standard test and MBCs were estimated according to Suarez et al. [11], with modifications. Pure broth cultures of *Bacillus sp.*, *Brachy bacterium sp.*, *Kocuria sp.*, and *Staphylococcus sp.* were grown overnight and absorbance was measured at 600 nm and diluted to 2×10^6 cells/ml. A mix culture with 2×10^6 cells/ml of each bacterial strain was also prepared. Stock solutions of the biocide were prepared and different dilutions were constituted. A volume of 500 µl culture was dispensed into each micro centrifuge tube, and biocide dilutions were added to get a final concentration of 500-8000 ppm. Duplicates were maintained. Positive controls of all cultures without biocides and negative controls (nutrient broth alone) were also maintained. All the tubes were maintained at 37°C for 24 hrs. From each tube, an aliquot of 200 µl was cultured on nutrient agar plates and incubated at 37°C. Bacterial colonies were counted.

Evaluation of the Selected Biocide by Coupon Weight Loss Method

The biocide (Predator 6000) was further evaluated by coupon weight loss method in five fuels (ATK, Diesel, Kerosene, Mo-Gas, and Ultra) to determine the effect of the biocide on bacteria and mild steel coupons. Custom made mild steel circular coupons of an average 2.45 cm diameter and 0.35 cm thickness and 13 g weight were grinded, smoothed and polished prior to use. Coupons were cleaned with distilled water, and then with acetone. These coupons were then grinded with silicon carbide papers (SiC) (Struers, Denmark) with a series of grit sizes; 220, 320, 500, 1000, 1200, 2500, and 4000. Distilled water was used in between and the coupons were grinded for 60 to 180s using each SiC paper placed on the turn table. They were grinded to a smooth and scratch free finish.

The coupons were further polished on a polishing device (Struers, Denmark). Polycrystalline diamond suspension (30 ml) (MetaD Supreme, Buehler, USA) ranging in sizes 15, 9, 6, 3, and 1µm was applied on a disc with nap surface (MD-NAP-T, Struers, Denmark) and each coupons were polished for 60 to 180 s. After polishing the coupons were wrapped in tissue paper and stored in a desiccator until use.

Coupon Weight Loss Experiment

The coupons were defatted in 95% alcohol for 15 min, air dried, and weighed. The coupons, flame sterilized with absolute alcohol, were incubated in each of the five fuels (50 ml) with mix culture (2×10^6 cells/ml of each bacterial strain) and the respective bactericidal concentration of the biocide for 15 d in glass flasks, with occasional mixing. A total volume of 300 µl mix culture in nutrient broth was added to each flask. Biocide control (w/o mix culture, with 300 µl nutrient broth) for the biocide and mix culture control (w/o biocide) and fuel control (w/o biocide, w/o mix culture, with 300 µl nutrient broth) were maintained. The experiment was done in duplicates. Post incubation, from each flask a volume of 200 µl was plated on nutrient agar to check the inhibition of bacterial growth. The coupons were rinsed with distilled water, incubated with 1N HCl for 30s to remove the corrosion products, wiped with paper towel, and air dried. To determine the percent weight loss, coupons were weighed [9] on semimicro balance (CPA225D; Sartorius, Germany) and calculated as follows:

% Weight loss =

$$\frac{(\text{Weight of coupon before incubation} - \text{Weight of coupon after incubation})}{(\text{Weight of coupon before incubation})} \times 100$$

Scanning Electron Microscopy of Coupons

These coupons were further analyzed for corrosion by electron microscopy (Analytical Scanning Electron Microscope; JSM-6010LA; Jeol).

Quality Analysis of Fuels after Treatment with the Biocide

Site was prepared at Kuwait Institute for Scientific Research campus and small model tanks (5 l) were installed in the field (Figure 2) for the storage of fuel samples (ATK, Diesel, Kerosene,

Mo-Gas, and Ultra) received from the oil sector in Kuwait. These are small-scale tanks made from same material (mild steel) used in Kuwait oil storage tanks, and these tanks are with outlets for easy sampling.



Figure 2: Mild steel tanks installed in the field.

Product quality analysis of ATK, Diesel, Kerosene, Mo-Gas, and Ultra after the addition of biocide was conducted at Petroleum Research Center (PRC), Ahmadi. The facility is equipped to conduct all of the required analyses according to ASTM and IP standard testing procedures. The Predator 6000, at its biocidal concentrations (2000 ppm) was added to 500 ml fuel and incubated at room temperature for 15 d with occasional mixing. Controls (without the addition of the biocide) were also maintained.

Density, American Petroleum Institute (API) gravity, color, water content, atmospheric distillation, and smoke point of fuels were analyzed and compared with the controls. Bacterial presence in fuels after treatment with the biocide was tested by culturing 200 µl in nutrient agar plates. Reference methods and equipment details are given in Table 1.

Parameter	Reference Method	Equipment
Density	IP190 (Energy Institute, 2005)	Density meter (Anton Paar GmbH, Austria)
API gravity	IP200 (Energy Institute, 2008)	Density meter (Anton Paar GmbH, Austria)
Color	D6045 (ASTM, 2012)	Spectrophotometric colorimeter (PFXi-195; Lovibond® Colour Measurement; Tintometer® Group, UK)
Water content	D6304 (ASTM, 2016)	Karl Fischer Titrator (71000 Aquamax Coulometric Karl Fischer Titrator; G.R. Scientific Limited, UK)
Atmospheric distillation	D86 (ASTM, 2016)	Aautomated distillation analyzer (OptiDist; PAC, USA)
Smoke point	D1322 (ASTM, 2015)	Smoke point analyzer (Smoke point-SP 10; Ad Systems, France)

Table 1: Reference Method and Equipment Used for the Parameters Studied.

Data Analysis

Inhibitory zone data was analyzed by linear regression. Data of the coupon weight loss experiment was analyzed by one-way Analysis of Variance (ANOVA) and data of the product quality experiments were analyzed by Anova: 2 factors. $p < 0.05$ was considered significant and the post hoc analysis was performed by Tukey HSD test.

Results

The biocide was screened for its inhibitory effect by agar well diffusion technique and was further studied to find MBC to weight loss experiment. The effect of biocide on mild steel coupons and different fuels were studied. Results of initial screening, MBC, effects of biocide on mild steel coupons, and product quality analysis are presented in the following sections.

Minimum Inhibitory Concentrations of the Biocide

The biocide (Predator 6000) was evaluated for the inhibitory effect on four bacterial strains and the mix culture.

In control plates without biocide, complete bacterial growth was observed. Inhibition zones were absent in lower concentrations, and diameter of the zones increased with increasing concentrations. Figure 3 shows linear regression of the squared inhibition zone radii (X^2) against the logarithm of the concentration of the predator 6000.

MIC of the biocide for each bacterial culture and mix culture, which are the antilogs of the zero intercepts of the regression lines, is presented in Figure 3. Predator 6000 was very effective against *Brachybacterium sp.* than the other bacterial strains (Table 2).

Biocide (ppm)	MIC for <i>Brachybacterium sp.</i>	MIC for <i>Bacillus sp.</i>	MIC for <i>Staphylococcus sp.</i>	MIC for <i>Kocuria sp.</i>	MIC for *Mix culture
Predator 6000	559.88	1308.93	2970.61	838.67	1927.10

Table 2: Minimum Inhibitory Concentration of the Biocide for *Brachybacterium sp.*, *Bacillus sp.*, *Staphylococcus sp.*, *Kocuria sp.* and Mix Culture.

*Mix culture: Equal proportions of the four bacterial strains *Brachybacterium sp.*, *Bacillus sp.*, *Staphylococcus sp.*, and *Kocuria sp.*

Coupon Weight Loss Experiment

In Diesel, Kerosene, and Mo-Gas, the biocide did not differ significantly ($P > 0.05$) with regard to coupon weight loss (Table 3). Coupons treated with the biocide (w/o mix culture) did not differ significantly when compared to coupons in fuel controls (w/o biocide, w/o mix culture). No bacterial growth was detected in the biocide treated fuels. Predator 6000 was completely soluble in all the fuels.

Scanning Electron Microscopy of Coupons

Pits corrosions were not observed on coupons after a 15 day treatment with the biocide and mix culture, in ATK, Diesel, Kerosene, Mo-Gas and Ultra (Figure 4).

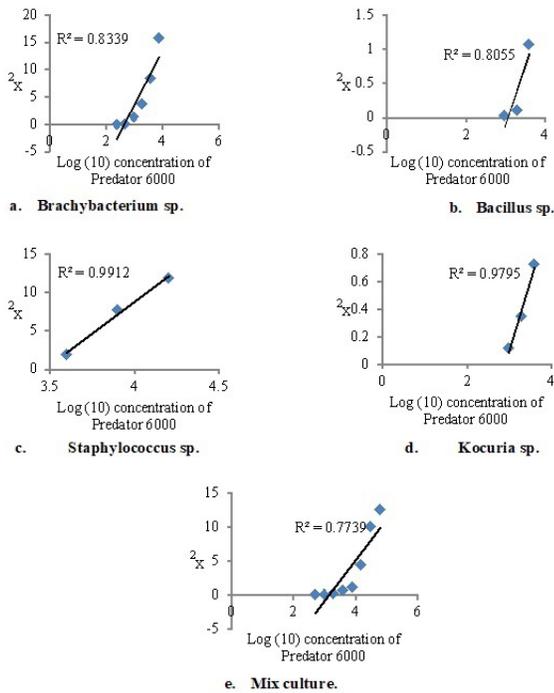


Figure 3: Linear regression of the squared inhibition zone radii (x2) against the logarithm of the concentrations of Predator 6000.

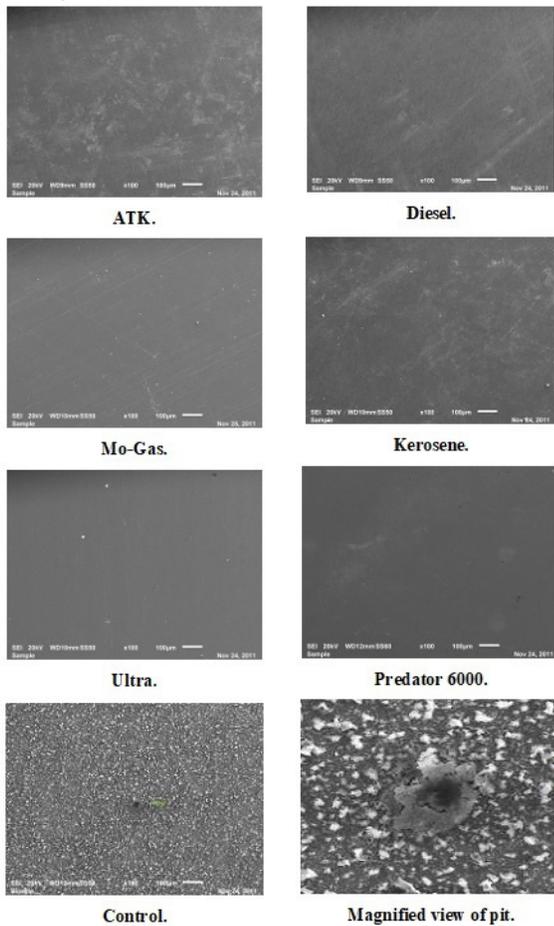


Figure 4: Scanning electron micrographs of coupon surfaces treated with predator 6000 in ATK, Diesel, Mo-Gas, Kerosene, Ultra, and Predator

6000 control w/o mix culture and with nutrient broth and control exposed to mix culture w/o biocide showing a pit and a magnified view of the pit.

Fuels	Predator 6000 + Mix Culture	Predator 6000 w/o Mix Culture	w/o Biocide w/o Mix Culture	w/o Biocide with Mix Culture
ATK	0.08 ± 0.017 ^b	0.13 ± 0.02 ^{ab}	0.25 ± 0.0066 ^{ab}	0.16 ± 0.0027 ^{ab}
Diesel	0.0025 ± 0.00025 ^c	0.01 ± 0.002 ^{bc}	0.2 ± 0.024 ^a	0.16 ± 0.015 ^{ab}
Kerosene	0.04 ± 0.016	0.041 ± 0.015	0.16 ± 0.0063	0.14 ± 0.073
Mo-Gas	0.27 ± 0.12	0.15 ± 0.15	0.31 ± 0.0086	0.24 ± 0.11
Ultra	0.07 ± 0.012 ^{bc}	0.027 ± 0.00034 ^c	0.27 ± 0.042 ^a	0.29 ± 0.06 ^a

Table 3: Percent Weight Loss of Mild Steel Coupons Treated with Mix Culture and the Biocide in Different Fuels.

Values are means ± standard deviation, n = 2 per treatment group. Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the TUKEY test.

All the fuel controls (w/o biocide, w/o mix culture, with 300 µl nutrient broth) were also corroded. Fig. 4g and h shows the corrosion in ATK control. Corrosion was very less in coupons treated with Predator 6000 containing fuels with mix culture (Figure 4a to 4f).

Quality of Fuels Treated with Biocide

ATK, Diesel, Kerosene, Mo-Gas, and Ultra were treated with the biocide Predator 6000 for 15 d and the details of fuel quality are given subsequently.

Density, API gravity, Color, and Smoke Point of Biocide Treated Fuels

Densities of the fuels treated with Predator 6000 (2000 ppm) did not show any significant difference (P>0.05) from the respective controls (Figure 5). API gravities (Figure 6) and color (data not shown) and smoke points (Figure 7) of ATK, Diesel, kerosene, Mo-Gas, and Ultra also did not change (P>0.05) after treatment with the biocide.

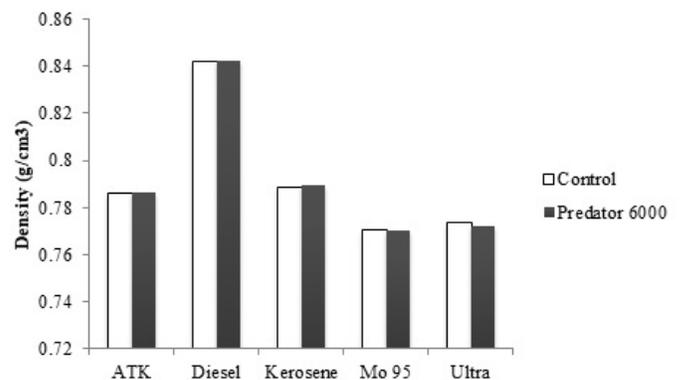


Figure 5: Densities of ATK, Diesel, Kerosene, Mo-Gas, and Ultra after treatment with the biocide.

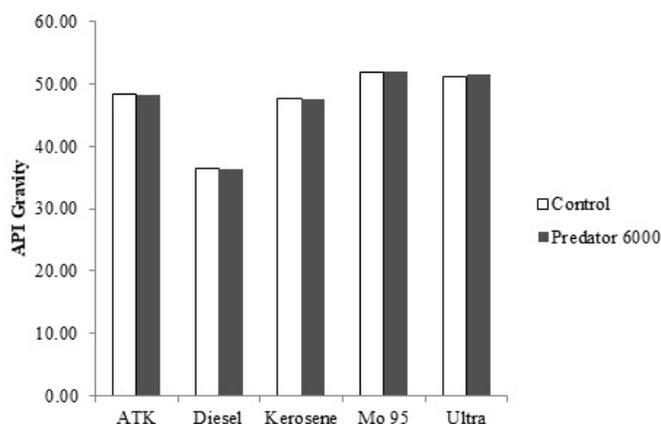


Figure 6: American Petroleum Institute gravities of ATK, Diesel, Kerosene, Mo-Gas, and Ultra after treatment with the biocide.

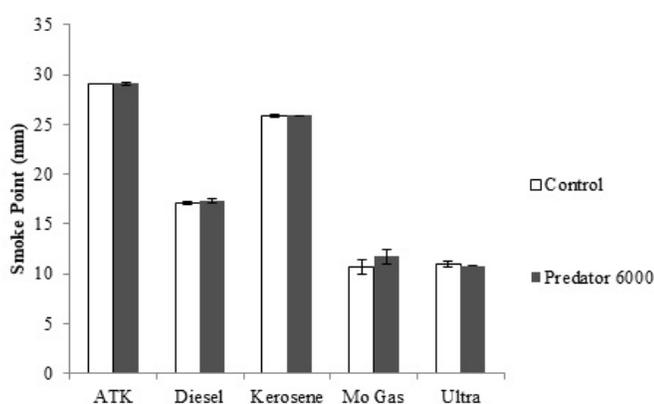


Figure 7: Smoke points of ATK, Diesel, Kerosene, Mo-Gas, and Ultra after treatment with the biocide.

Water Content of Biocide Treated Fuels

Water content of all the five fuels increased ($P < 0.05$) after the addition of the biocide (Table 4). In ATK, water content increased from 22 ppm to 43 ppm from control after treatment with the biocide. In diesel and Mo 95, the lowest increase in water content was in Predator 6000 treatment, whereas Kerosene had similar increase in water content after treatment with Predator 6000 (27 ppm). When compared to the control, water content in ultra-increased by 210 ppm, after treatment with Predator 6000 (Table 4).

Fuels	Control (ppm)	Predator 6000 (ppm)
ATK	32.97	75.82
Diesel	48.94	67.09
Kerosene	33.48	60.35
Mo 95	286.08	355.81
Ultra	170.17	380.47

Table 4: Water Content of ATK, Diesel, Kerosene, Mo-Gas, and Ultra after Treatment with the Biocide.

Atmospheric Distillation of Biocide Treated Fuels

Atmospheric distillation results of ATK (Table 5), Diesel (Table 6), Kerosene (Table 7), Mo-Gas (Table 8), and Ultra (Table 9)

are given in this section. Atmospheric distillation temperatures of the fuels did not change considerably from the controls. In ATK, maximum difference was in 95% distillation of Predator 6000 treatment, which showed a decrease in 4.4 °C. Initial boiling Point (IBP) and final boiling point (FBP) did not change considerably (Table 5). 95% distillation of diesel showed an increase of 12.9 °C in Predator 6000 treatment (Table 6).

Atmospheric Distillation	Control (°C)	Predator 6000 (°C)
IBP*	154.1	153.4
5%	163.5	163.5
10%	166.0	166.4
15%	168.6	168.4
20%	171.5	171.1
30%	175.3	175.3
40%	180.8	180.5
50%	186.9	186.5
60%	194.0	193.7
70%	202.4	201.9
80%	213.0	212.1
85%	220.0	218.6
90%	229.2	227.2
95%	246.0	241.6
FBP*	262.5	261.8

Table 5: Atmospheric Distillation Results of ATK Treated with Predator 6000. *IBP – Initial boiling point; FBP – Final boiling point

Atmospheric Distillation	Control (°C)	Predator 6000 (°C)
IBP*	209.3	206.6
5%	238.3	236.8
10%	247.4	245.7
15%	254.3	252.1
20%	259.0	258.1
30%	269.7	269.5
40%	280.2	280.2
50%	289.6	290.3
60%	299.4	301.1
70%	310.4	313.2
80%	322.6	327.3
85%	329.3	335.7
90%	337.6	346.1
95%	347.8	360.7
FBP*	374.6	374.5

Table 6: Atmospheric Distillation Results of Diesel Treated with Predator 6000. *IBP – Initial boiling point; FBP – Final boiling point.

Atmospheric Distillation	Control (°C)	Predator 6000 (°C)
IBP	155.6	154.4
5%	169.7	170.2
10%	174.8	175.0
15%	178.3	178.4

20%	181.2	181.3
30%	186.6	186.8
40%	191.8	191.7
50%	197.3	197.2
60%	203.4	203.3
70%	210.5	210.6
80%	219.1	219.1
85%	224.2	224.3
90%	230.6	230.5
95%	239.1	239.2
FBP	254.9	253.5

Table 7: Atmospheric Distillation Results of Kerosene Treated with Predator 6000. *IBP – Initial boiling point; FBP – Final boiling point.

Atmospheric Distillation	Control (°C)	Predator 6000 (°C)
IBP	41.5	40.7
5%	61	60.9
10%	67.1	67.2
15%	71.9	72.1
20%	76.6	76.9
30%	86.4	87.5
40%	98.6	99.5
50%	109.8	109.7
60%	120.9	121.9
70%	133.1	134.1
80%	145.9	147.1
85%	153.2	154.9
90%	162.4	165.1
95%	178.5	184.3
FBP	188.6	184.3

Table 8: Atmospheric Distillation Results of Mo-Gas 95 Treated with Predator 6000. *IBP – Initial boiling point; FBP – Final boiling point.

Atmospheric Distillation	Control (°C)	Predator 6000 (°C)
IBP	37.7	37.2
5%	58.2	56.3
10%	66.3	64.0
15%	72.5	70.2
20%	78.5	76.0
30%	90.7	87.7
40%	104.4	101.1
50%	114.5	111.4
60%	125.4	122.8
70%	135.8	132.9
80%	146.5	143.9
85%	151.3	149.4
90%	160.8	157.3
95%	179.7	170.4
FBP	186.5	189.1

Table 9: Atmospheric Distillation Results of Ultra Treated with Predator

6000. *IBP – Initial boiling point; FBP – Final boiling point.

IBP decreased by 1.2°C in Kerosene after treatment with Predator 6000 (Table 7). In Mo-Gas, 95% distillation temperature increased in Predator 6000 by 5.8 °C treatment (Table 8). IBP of Ultra decreased by 0.5°C after treatment with Predator 6000 (Table 9), whereas FBP increased by 2.6°C after treatment with Predator 6000. No microbial growth was detected in any of the fuels after treatment with the biocide, and a few colonies were detected in the controls w/o the biocide.

Discussion

Microbiologically induced corrosion is a major problem in the fuel storage tanks used in the oil industry. Microbiological corrosion is induced by bacteria, fungi, and algae [12,13]. For prevention and control of microbiologically induced corrosion, the system needs to be clean [2,12,14], which is not always practical [2]. Application of biocides can keep corrosion under control. Biocides can be either oxidizing or non-oxidizing, of which non-oxidizing are more effective. Non-oxidizing biocides are usually formaldehyde, glutaraldehyde, isothiazolones, and quaternary ammonia compounds [2].

The Predator 6000 is a mixture of 4-(nitrobutyl) morpholine (60-100%) and N,N'-methylenebismorpholine (4.99-9.99%). Even though it releases small amounts of formaldehyde, this will not contribute to substantial antimicrobial effect [8]. Oil soluble corrosion inhibitors such as Predator 6000 may form a persistent monolayer film adsorbed at the metal solution interface, which prevents corrosion [15].

The MIC of an antibacterial agent for a given organism is the lowest concentration of the agent required to inhibit the growth of an inoculum of the bacterium in a standard test. The MBC is the minimal concentration of antibiotic that kills the inoculum and can be determined by sub-culturing to agar media without antibiotics. MBC is the minimal amount of antibiotic that results in a ≥99.9% decrease in the initial inoculum within 24 h in a standard test [16]. MBC of the Predator 6000 is 2000 ppm. MIC for Predator 6000 ranged from 560 to 2971 ppm of the product for pure cultures.

The coupon weight loss experiment demonstrated that corrosion in Predator 6000 coupons was very less when compared to the controls. This biocide was completely soluble in all the fuels tested. This result is in contrast with the view that isothiazolone mixture and quaternary ammonium compounds are more effective than glutaraldehyde and formaldehyde releasing agents [3,4].

Quality of the all the five fuels (ATK, Diesel, Kerosene, Mo-Gas, and Ultra) did not change in terms of density, API, color (data not shown), and smoke point after treatment with the biocide (Predator 6000). But water content of all the fuels increased considerably when treated with each of the biocide. Use of biocide in powder form may solve this problem. Atmospheric distillation temperatures of the fuels increased or decreased slightly in some cases but not much as to affect the performance of the fuels.

Conclusions

Inhibitory effects of the biocide was screened against a mix culture of *Bacillus sp.*, *Brachy bacterium sp.*, *Kocuria sp.*, and *Staphylococcus sp.* by agar well diffusion technique, and MICs were estimated. The biocide (Predator 6000) was further studied to find its effect on the quality of fuels (ATK, Diesel, Kerosene, Mo-Gas, and Ultra) and on the mild steel coupons. Quality of the all the five fuels did not change in terms of density, API, color, smoke point, and atmospheric distillation, after treatment with the biocide (Predator 6000). Water content of the fuels increased after treatment with the biocide.

Coupon weight loss study proved that the biocide did not change the weight of the coupons significantly. But coupons in fuel controls (w/o biocide, w/o mix culture, with 300 µl nutrient broth) were corroded. Interestingly, Predator 6000 showed very less degree of corrosion when compared to fuel controls. So Predator 6000 can be considered as a very effective biocide against the bacteria in the fuel storage tank in Kuwait.

References

1. Sondossi M. Biocides Non-public health, nonagricultural microbials. In Eds Schaechter, M, Lederberg J. The Desk Encyclopedia of Microbiology. Oxford, UK: Elsevier Academic Press. 2004; 147-160.
2. Videla HA, LK Herrera. Microbiologically influenced corrosion: Looking to the future. *International Microbiology*. 2005; 8: 169-180.
3. Allsopp D, KJ Seal, CC Gaylarde. Introduction to Biodeterioration. Cambridge. Cambridge University Press. 2004.
4. Yemashova NA, VP Murygina, DV Zhukov, et al. Biodeterioration of crude oil and oil derived products: A review. *Reviews in Environmental Science and Bio/Technology*. 2007; 6: 315-337.
5. Rossmoore HW, JW Wireman, LA Rossmoore, et al. Factors to consider in testing biocides for distillate fuels. In *Distillate Fuel: Contamination, Storage, and Handling*. Edited by Howard L Chesneau and Michele M. Dorris. Philadelphia: ASTM International. 1988.
6. Gaylarde CC, FM Bento, J Kelley. Microbial contamination of stored hydrocarbon fuels and its control. *Revista de Microbiologia*. 1999; 30: 01-10.
7. Paulus W. Directory of Microbicides for the Protection of Materials: A Handbook. Netherland: Springer Science & Business Media. 2005.
8. Zielinski RE, MA Christa Chilson. Antimicrobial additives for metalworking lubricants. In *Lubricant Additives: Chemistry and Applications*. Edited by Rudnick, L.R. Florida: CRC press. 2009.
9. Mataqi K, Q Al-Matawah, A Akbar, et al. Assessment and control of biomass growth in fuel storage tanks in Kuwait-Phase I: Quantification and identification of microbial activities in the products storage tanks. Kuwait Institute for Scientific Research, Report No. 11069, Kuwait. 2012.
10. Bonev B, Hooper J, Parisot J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *Journal of Antimicrobial Chemotherapy*. 2008; 61: 1295-1301.
11. Suarez M, JM Entenza, C Doerries, et al. Expression of a plant-derived peptide harboring water-cleaning and antimicrobial activities. *Biotechnology and Bioengineering*. 2003; 81: 13-20.
12. Lane RA. Under the microscope: Understanding, detecting, and preventing microbiologically influenced corrosion. *Journal of Failure Analysis and Prevention*. 2005; 5: 10-12.
13. Little BJ, PA Wagner, F Mansfield. Microbiologically Influenced Corrosion. Houston, TX: NACE International. 1997.
14. Videla HA. Prevention and control of biocorrosion. *International Biodeterioration & Biodegradation*. 2002; 49: 259-270.
15. Rajasekar A, Maruthamuthu S, Palaniswamy N, et al. Biodegradation of corrosion inhibitors and their influence on petroleum product pipeline. *Microbial Research*. 2007; 162: 355-368.
16. French GL. Bactericidal agents in the treatment of MRSA infections-the potential role of daptomycin. *Journal of Antimicrobial Chemotherapy*. 2006; 58: 1107-1117.