Research Article

Indian Journal of Microbiology Research

ISSN 2394-546X(Print)
e-ISSN 2394-5478(Online)

IJMR

DETERMINATION OF THE SEVERITY OF MICROBIAL CONTAMINATION IN DIESEL FUEL OF A STORAGE TANK.

Prahlad Rangra[1], Tina Chaturvedi[2], Amit Bhattacharya[3*]

[1,2,3]Dow microbial Control, Dow Chemical International Pvt. Ltd. Plot no.8A/1, 2 Kalwa Block, Thane Belapur Road, Navi Mumbai, Maharashtra

***Correspondence Author**
E-mail: abhattacharya@dow.com

Abstract
Background: *Hydrocarbon degrading microorganisms has been reported from fuels including diesel (1). Current literature suggests that microbial species can be metabolically active in complex hydrocarbon and degrade up to 66% of diesel oil in just 28 days (5). In this study the diesel samples collected from storage tank were found heavily contaminated with microorganisms which are already reported as potential biodegrades.*
Method: *Detection of the microbial contamination was done by serial dilution plating on R2A for aerobic bacteria, PDA for fungal and Sulphate API agar for anaerobic bacteria. Further isolated bacterial species was identified by Biolog system.*
Results: *Diesel samples were found heavily contaminated with species known for degrading oil and its products.*
Conclusion: *This study has proved that diesel oil is highly prone to the microbial contamination which can lower down the product performance to a great extant therefore; the need is to prevent diesel oil from getting contaminated with the microbial species. Addition of high quality and environment friendly biocides is important to prevent microbial growth in Diesel.*

Keywords: *Diesel oil, Biocides, Corrosion, Contamination, Biolog.*

Background

Refineries are one of the largest industries of the world. Every year a huge amount of the money is spent to modify, modernize the installations of the refineries. Microbial contaminations to these equipments shorten the life of costly assets and deteriorate product quality including diesel. Data about various research institutes had already made our understanding easy that microbial population has the potential of degrading hydrocarbon products. It is reported that ppm levels of water content is found in diesel, which is after condensation form a layer below the diesel being higher density. This water at the bottom is main cause for onset of microbial contamination and deterioration of hydrocarbon i.e diesel. This contamination if transferred to the fuel lines and filters injectors of the automobiles may chock them and create pressure built.
Variety of additives used to improve the stability of the fuels such as aliphatic amines, chelating agents, detergents and corrosion inhibitors can act as a nutrient source for microorganisms.

In most of the storage tanks oxygen is normally present in sufficient quantities for the aerobic microbes, and is continually replenished when tanks are refilled. However, even if the fuel becomes anaerobic, it is not protected from microbial attack,

since facultative organisms, such as *Bacillus*, and anaerobes, such as sulfur-reducing bacteria (SRB), continue to thrive. The factors of microbial growth are probably availability of minerals, particularly phosphorus, which is generally present at less than or equal to 1ppm in the fuel. Nitrogen and iron may also be important limiting nutrients. In diesel fuel, microbial contamination not only decreases the life expectancy but also induce corrosion to the pipelines, storage tanks and formation of the bacterial biofilms. As said above this can later block the pipeline, filters and pumps. The present study aimed for isolation of diesel degrading bacteria from sample collected from the storage tanks.

Materials and Methods

Two well agitated diesel samples were collected from storage tanks. Agitation was done so as to quickly distribution of the microbial population to entire tank so that exact count per ml can be achieved. After agitation samples from bottom sampling point were collected in a sterile vial in as aseptic conditions as possible and used for the further identification.

Sample Processing and Culturing:

Collected samples were centrifuged at 3000 rpm/ 30 mins so as to get traces of water and pallet at the bottom of the centrifuge tubes. Pallets were then allowed to redistribute in autoclaved distilled water, which further used for microbial enumeration. Theses samples were now serially diluted to 10^3 dilution and 0.1ml of each dilution plated on the R2A and PDA (Himedia) agar media. Also original samples were also plated on the Sulphate API agar media (Himedia) to know the presence of the anaerobes or any facultative anaerobes.

After 72 hour incubation data count was taken and single isolated colonies were again streaked on R2A plates so to get pure isolated cultures of bacterial colonies for characterization.

Identification of Aerobic and Anaerobic Bacteria Using Biolog:

Biolog is a simplified system used for the characterization of microorganism on the basis of pattern generated by metabolism of carbon substrates. Carbon substrates are already placed in dehydrated form in 96 well plates (ready to use plates), out of which 95 wells contains carbon substrates and 1 blank well. For the characterization of microbes, fresh overnight grown culture was inoculated in the inoculation fluid (IF-A, IF-B for aerobes and AN-IF for anaerobes) with described turbidity. Turbidity was set by turbidometer supplied by Biolog Inc. after turbidity adjustment set solution was transferred to trough and 100 µl of the solution was suspended in the each well (GEN III plates for aerobes, AN plate for anaerobes). Plates were then incubated in aerobic and anaerobic chamber for 24-48 hours. Metabolism of the carbon sources is indicated by reduction of tetrazolium violet dye that results in the appearance of purple color. After incubation plates were scanned under microplate reader connected with computer system having microlog 3 software. This software was used to compare the metabolism pattern of the test organism with the data pattern present in Biolog database. Results were displayed according to the similarity index (bacteria showing closest similarity metabolism pattern with the pattern generated by test organism).

Observation and Results

Diesel samples showed the presence of sludgy water droplets which indicated about the possibility of conderable number of microorganisms in it. The CFU count was found to be 10^6 cfu/ml for aerobic bacteria, 10^4 cfu/ml for fungal species and 10^3 for the anaerobic bacteria

Figure 1 dilution plated Diesel sample.
R2A Plate shows colonies obtained after dilution plating.

The bacterial species were purified on solid plates (Fig 2).

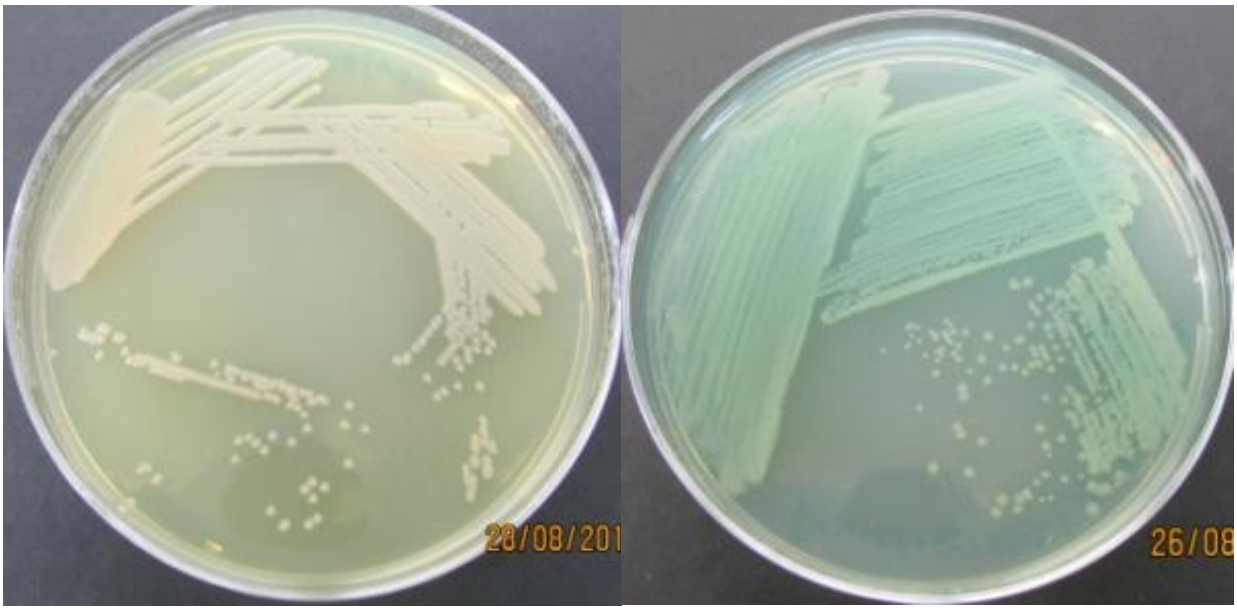

Figure 2 Purified diesel isolates.
The isolates were purified on R2A media by streak plate method.

Purified cultures then subjected to biolog system which showed the similarity index of 0.653 for *Pseudomonas aerugenosa* and 0.510 for *Bacillus sp.*, similarly purified Anaerobic bacteria when subjected to Biolog Characterization it showed *Veillonella sp.* with similarity index of 0.192. In Biolog identification any similarity index value ≥ 0.5 gives reliable identification up to species level.

Discussion

The organisms isolated from diesel sample in this study have been confirmed by other workers to have hydrocarbon degrading abilities. Sample studied by plating technique showed high count of bacteria, fungi and anaerobic bacteria. Purified aerobic and anaerobic bacterial cultures were identified as *Pseudomonas aerugenosa*, *Bacillus* sp, *Veillonella* sp. These bacteria are already reported for the degradation of complexed hydrocarbons present in the fuels (1,4,6,7). It has been observed that in the fuel storage tank, water is condensed and accumulated at the bottom. Variety of micro-organism grows in the water and eventually degrades hydrocarbons as nutrient at interface. Ijah and Antai (2003) reported *Bacillus* spp. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples (30 and 40% crude oil) (3).

Singh and Lin in 2008 reported *Bacillus pumilis*, *Acinetobacter calcoaceticus, Citrobacter freundii* in which *Bacillus pumilis* was found to degrade approximately 87% diesel in just 2 weeks (8). Nisha et al. (2013) isolated 5 pure cultures, 2 *Staphylococcus sp*, *Pseudomonas sp*, 2 *Bacillus sp* from diesel contaminated soil and found that all these bacteria were able to degrade diesel (5).

Conclusion

Microbiology is still a rudimentary subject to Oil and Gas industries especially in refineries. Refineries stock up a substantial amount of diesel in their storage tank before transferring to their customers. During storage the water accumulates at the bottom and organize right platform for microbial growth. This bacterial growth in the bottom of diesel lowers down the quality of diesel substantially in due course of time. Presence of the microbes in storage tanks and also in the transfer lines tends to generate biofilm which induces corrosion.

Various reports have shown that the microbes observed in the samples have good potential in degrading diesel oil, such as for *Pseudomonas aerugenosa* it is 66 % and for *Bacillus* sp. 16% in 30 days (4, 7). Similar to these observations, our study also confirmed the presence of *Veillonella*, *Bacillus* and *Pseudomonas* in diesel and were identified by Biolog system up to genus level and species level respectively. Degradation of diesel causes detrimental effect in performance and therefore, should be avoided. Although the best would be prevention but once system gets microbially contaminated, use of appropriate preservatives becomes important. Selecting a right candidate for preservation of diesel should have following criteria

- No effect on fuel properties.
- Efficacy against broad spectrum microorganism.
- Compatibility with the other fuel additives.
- Environmental friendly biocides.
- Cost effectiveness.

With the better understanding of the microbiology of refinery products (diesel) and their metabolism can help to improve product quality and its market value.

List of Abbreviation

CFU: - Colony forming unit.
SRB: - Sulphate Reducing Bacteria.
R2A:- Reasoner´s 2A agar.
PDA: - Potato dextrose agar.
IF: - Inoculating fluid.
AN-IF: - Anaerobic inoculating fluid.
HUM-Bug: - Hydrocarbon using microorganism.
H_2S:- Hydrogen sulphide

Acknowledgement

We are thankful to TATA telecommunication, Mumbai for providing us diesel sample.

References

1. Adesanya O.O, Osho A, Durugbo E, Akinyemi O, Shokunbi O: **Hydrocarbon degradation potentials of bacterial species isolated from bitumen contaminated water and sediments in Ilubirin, Temidire camp, and Agbabu communities of Ondo state, South West Nigeria.** Journal of international academic research for multidisciplinary 2014, **2(5)**: 239-248.
2. Bookam P, Bhattacharya A: **Isolation and identification of sulphate reducing bacteria from spoilt paint.** Paintindia 2014, **64(4)**:64-68.
3. Ijah UJJ, Antai SP. **Removal of Nigerian light crude oil in soil over a 12-month period** 2003. Int. Biodeterior. Biodegradation. **51**: 93-99.
4. Nwaogu L A*, Onyeze G. O. C, Nwabueze R. N: **Degradation of diesel oil in a polluted soil using *Bacillus subtilis*.** African Journal of Biotechnology 2008, **7 (12)**:1939-1943.
5. Nisha P, Nayana M, Varghese V: **Degradation Studies on Diesel Oil Using Bacterial Consortium Isolated from Oil Polluted Soil.** Advanced Biotechlogy 2013, **13(02)**:8-14.
6. Rovery C, Etienne A, Foucault C, Berger P, Brouqui P: ***Veillonella montpelliérensis* Endocarditis.** Emerging Infectious Disease journal 2005, **11 (7)**: 1080-6059.
7. Sharma A, Kumar P, Rehman BA: **Biodegradation of Diesel Hydrocarbon in Soil by Bioaugmentation of *Pseudomonas aeruginosa*: A Laboratory Scale Study.** International Journal of Environmental Bioremediation & Biodegradation 2014, **2(4)**:202-212.
8. Singh, C, Lin J. **Isolation and characterization of diesel oil degrading indigenous microrganisms in Kwazulu-Natal, South Africa.** African Journal of Biotechnology 2008, 7 (12): 1927-1932.

How to cite this article: Rangra P, Chaturvedi T, Bhattacharya A Determination of the severity of microbial contamination in diesel fuel of a storage tank. Indian J Microbiol Res 2014;1(1):72-76.