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Degradation of Lubricant Oil Residues: The Role of Rhizosphere Microflora

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***Abstract*-Lubricant oil residues are potentially harmful, toxic and exhibit considerable persistence. The intrinsic components which are mostly oxidation products are also highly dangerous to plant and animals of land as well as of aquatic origin. Burning of used lubricant oil leads to a major cause of air pollution. The answer to such problems is utilizing microbes via bioremediation. Literature is available on isolation of microbes from the lubricating oil used in engine of vehicles. The spent oil is discarded as waste and periodic replenishment of fresh oil in engines is a practice. There are reports available that relate to creating a consortium of microbes and using them for degradation of lubricant oil. In this present review, importance is given on using soil rhizosphere microflora in degradation of lubricant oil, given the potential edge rhizosphere microflora exhibits over non-rhizosphere microbes.**

***Keywords* - Lubricant oil, rhizosphere, biodegradation, analysis**

I. INTRODUCTION

A lubricant is accepted as a substance which is generally used to reduce friction between two surfaces or to help in transport of materials. This property, by which lubricant oil reduces friction, is called lubricity [1]. Although indispensable, it becomes a major case of toxic waste when spent or disposed. Different ways of treating this type of toxic waste are recycling or reduction of use, degradation and chemical transformation. 13% (EC countries) and 32% (USA) of all used lubricants are given out into the environment more or less similar in chemical properties [2]. The reason is that lubricant oil is not very often treated or detoxified, but given out into the environment by leaks and careless disposal. Used oil handling also poses certain grave problems, which calls for a well-documented system in the different companies which deal with lubricant oil manufacture and trade [3].

A. LUBRICANT OIL

The most common application of lubricant oil is being used as motor oil in engine of vehicles. It provides a safe environment for the internal combustion engines by reducing friction, carrying away contaminants, protection against wear and corrosion. Classically lubricants contain 75-80% base oil. This is nothing but petroleum fractions, also called as mineral oil. It goes up to 99% in industrial lubricants [4]. Additives are also added which range from 5-30% [5]. Vegetable oil such as silicones, hydrogenated polyolefins, esters and fluorocarbons are sometimes used as base oils. Additives are required to reduce friction and corrosion, to improve viscosity, lessen the degree of oxidation, and reduce contamination [1].

B. LUBRICANT OIL COMPOSITION

The American Petroleum Institute, commonly referred to as API, classifies the types of lubricant base oil [1] as:
Less than 90% are saturates. Sulphur content can be more than 0.03%, and viscosity index (VI) of 80 to 120. This is denoted by SAE or Society of Automotive Engineers as Group I.
When saturates are over 90% and sulphur content under 0.03%- Group II.
When saturates are over 90%, sulphur below 0.03% but viscosity index is over 120- Group III.
Polyalphaolefins (PAO) containing base oil is clubbed under Group IV.

Base oil may be seen to contain polyalkylene glycol, naphthenics or esters. This comprises Group V.

Based on the composition, lubricant oil can also be classified as paraffinic, naphthenic and aromatic [1]. Lubricant oil is made from either petroleum based or non-petroleum based sources and mainly, base oils are made of hydrocarbons or polyalphaolefins. One type of base oil is used commonly, but more than one type can also be used for better performance of the engine [6, 7]. It is seen that high performance base oils have esters up to 20% by weight, the reason being their ability to provide excellent lubricity [8].

C. ADDITIVES

In addition to base oil, certain chemical compounds are required to enhance the performance of engines. These are called as additives. The manufacturer can opt for the same formulation for base oil and can choose different additives based on the required applications. Some of the important classes of additives are detergents, antioxidants, corrosion and rust inhibitors, friction reducers, and agent for viscosity index and stickiness improvement [5]. Using the test system “O_2/CO_2 Headspace Test with Gas Chromatography and Thermal Conductivity Detector (GC-TCD)” effect of additives on lubricant oil biodegradation can be checked. The principle lies in the measurement of carbon dioxide and water obtained by final aerobic mineralization of substances [9]. It is seen that biodegradability is not highly influenced by the presence of additives. However, toxicity is affected only by additives [9].

D. CONSUMPTION OF LUBRICANT OIL

According to 2005 statistics, India uses a total of 3876000 tons of lubricant oil for non-energy purposes. In 2009, India was at 29,80,000 bbl/day per 1000 people [10,11]. In 2010, USA consumed the maximum amount of lubricant oil [11]. China and India rank second and third respectively. India holds responsible for 5.3% of the total global demand owing to increased number of vehicles and exploding population [12].

II. ENVIRONMENTAL IMPLICATIONS OF LUBRICANT OIL

Lubricants are indispensable in industries and vehicles, but the usage comes with potential harm to the environment. Discharge into water bodies or drainage systems or simple waste on to barren soil are commonly seen. A slightly more on the rarer face are accidental spillage, pipeline leakages, man-made or natural disasters [13]. Though effects of such disposal may appear not of immediate concern, in the long run it contributes to the deterioration of environment on the whole. Around 600000 tonnes of total lubricants is given out into the environment carelessly, every year, in the European Union alone [14]. The safest way to treat any environmental hazard is to seek solutions in the environment itself. While exploring the different ways to bioremediate any toxic waste or pollutant, it is seen that soil microflora provides a variety of microbial species with powerful degrading capacity. In particular, the microbes that inhabit the rhizosphere region exhibit metal and oil degrading abilities. Using such microbes a safe way to manage lubricant oil pollution can be found. Environmental hazards are an offshoot of technology, but due to advances in the same field it is possible to evaluate effects and risks before introducing any compound into the environment [4]; this can be applied to lubricants too [2,15].

A. THE RHIZOSPHERE

Rhizosphere, a term introduced by German scientist Hiltner in 1904, is the zone of soil bordering the root system of plants. The zone is around 1mm wide but cannot be distinctly distinguished. It is the area which exhibits immense biological and chemical activity. The region surrounding the roots can be distinguished into many microhabitats [13]. The root exudates and the microbes feeding on the exudates are responsible for such diverse biochemical and metabolic activities. The microbes too provide nutrients to the plant in a mutual manner. These interactions of plants and microbes make the rhizosphere the most vibrant environment in the soil with higher capacity to degrade different toxic compounds [3]. One such interaction is provided by strigolactones, which are plant hormones that help in association between plant and fungi. Bacteria which are capable of fixing nitrogen demonstrate diurnal cycles and are seen to be present in rhizosphere of rice. These mimic plant behavior, supplying direct proportion of nitrogen to its demand [16].

B. THE RHIZOSPHERE EFFECT

The overall impact of plant root system on the microbes in soil represents the term “rhizosphere effect”. Distinguishing factors of rhizosphere from bulk soil are biophysicochemical processes occuring due to uptake of water and nutrient [17, 18]. Most abundantly seen microbes are bacteria and fungi [19]. The bacterial fraction can be in a range of 10^9-10^{10} cells g^{-1} of soil or within a variation of 10^6 cells mm^{-3} in the rhizosphere biofilm [16]. Different exudates liberated by plants also vary depending on associated microbes and soil type, moisture content in soil, pH, temperature, along with age of plant. One of the contributing aspects to higher breakdown of organic compounds is enzymes such as oxidoreductases, hydrolases, lyases and transferases secreted by both plant and microbes. The intensity of microbial activity depends on the exudates released by them and the distance of migration [16]. Naturally, rhizosphere microflora has been studied and enumerated exhaustively by culturing, microscopic and biochemical characterization [20]. Most commonly seen microbes are:

Bacteria**:** Greater rhizosphere effect is seen with bacteria than fungi. Predominant are Gram negative, rod-shaped, non-sporulating bacteria like *Pseudomonas* and *Agrobacterium*. Most common genera present are *Athrobacter, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas, Micrococcus* etc. Aerobic bacteria are not present abundantly due to lower levels of oxygen.

Fungi: The differences seen in fungal growth in field and in culture media is due to the presence of spores giving inconsistent results in culture media, thus misleading results. *Fusarium, Penicillium, Aspergillus* and *Verticillium* show stimulated growth in rhizosphere soil.Lower fungi such as *Phytopthora, Pythium, Aphanomyces* are strongly associated to plants.

Actinomyces, Protozoa and Algae:Rhizosphere effect on Actinomyces has not been elaborately defined as it is found that Actinomyces are influenced to a much lesser degree. *Nocardia, Streptomyces* play a significant role. Protozoa, flagellates govern and ciliates are atypical.

III. ANALYSIS OF DEGRADATION OF OIL

The foremost necessity in analysis of biodegradability is to choose the right technique for analysis of the same. Further studies can be carried only on the basis of the results obtained by the technique applied. The choice depends on sample used and also the conditions under which biodegradability is being studied. A potential problem which rises is poor water solubility of lubricant oil. To minimize the effect of hydrophobic properties of oil, model systems of water-soluble substances can be developed. Aqueous systems can also be used, which are dispersions of poorly water-soluble substances [4, 21]. These solutions must be homogenized to lower hydrophobic effect of oil [4]. Most important methods in practice are Fourier Transform Infrared Spectroscopy [22], High-Pressure Differential Scanning Calorimetry [23] and Bulk Oil Oxidation Stability Test (18). Commonly used but laborious processes are checking the Total Acid and Total Base, which are referred to as TAN (Total Acid Number) and TBN (Total Base Number). Insoluble Content can also be measured, designated as IC. International Organization for Standardization (14593 test), combined with Economic Co-operation and Development (OECD test 310), is a useful tool which can be applied under practical circumstances for research and ecological testing [4]. Lubricant oil with branched structure of acid residue in base oil shows 90% biodegradability as revealed by OECD 310 (ISO 14593) test [4]. Properties such as oxidation duration (OD), TAN and IC determine the rate of oil degradation and can be determined by Dielectric Spectroscopy [24], coupled to Partial Least Square (PLS) multivariate calibration. Advantage is that the results obtained are not only precise but also quantitative. Temperature, too, plays an important role and it was seen that higher temperature (100ºC) was more enhancing. Electrochemical Impedance Spectroscopy (EIS) and Non-Linear Electrochemical

Impedance Spectroscopy (NLEIS) connect electrochemical properties and lubricants [25]. This is done by relating electrochemical properties to significant physical and chemical traits of oil. Using Dielectric Spectroscopy to study OD, TAN and IC [24] three virgin oils were used as three sets. The brands used were KunLun (China), Shell and Dalian Petroleum (China). The oxidation temperature for each was 150°C. Analysis of these three properties was done using the three different oils. The values were recorded according to sampling time. It was seen that OD increases with time, starting from 0 at the first minute to 8945 at the 11th reading. Similarly, TAN also increases, dropping once at the 5th reading. IC was shown to have a random data generated, with highest recorded at 5.60 mg/g. This was for set one, that is, for the first type of oil. For set two, OD was again seen to gradually increase with sampling time. But both TAN and IC showed variable results. TAN initially decreased and then it increased at 9th reading. IC too, showed a major increase after 9th reading. Similar results were obtained for set three.

To check the biodegradability of different types of oil, a respirometry method developed by Bartha and Pramer can be chosen [20]. It is a device used to measure the CO_2 formation during degradation of oil and kinetics can be developed by employing mathematical models. Results show that used engine oil is easily biodegradable than fresh oil, since their structures have already been compromised with due to high temperature inside the engine. Higher rates of CO_2 formation is observed in used oil and automotive oil rather than vegetable oils [20]. Broadly, degraded oils have higher amounts of acid along with metals such as Fe, Cr, Cd and Pb [26].

Another technique to be considered is NMR Relaxometry. A blending of techniques such as ^{13}C NMR with ^{1}H NMR spectroscopy has been used to determine methylene, ethylene and quaternary carbon components [27, 28, 29], using Larmor frequency, which is the angular frequency for an external field axis. Dispersion studies were done to find possible correlation between various processes which arise during degradation and consequent effects at the molecular level [5]. It depends on the ageing effects in lubricant oil, observed in proton T_1^{-1} dispersion, significant effects seen at low Larmor frequencies. It was concluded that for samples which have undergone degradation, relaxation becomes more coherent, which means higher T^{-1}. Also, the contrast between spent and fresh samples becomes more discernible at low frequencies. This suggests that there is an intense relation between degradation and molecular dynamics modifications. But of course, the degradation mechanism cannot be predicted by such experiments. The differences are also dependent on the oil formulation. It is applicable to say that NMR provides us with a ‘fingerprint’ of a fresh formulation.

A sensor has been designed for immediate measurement of grade of used and un-used oil [30]. This is an economically feasible monitoring system. The methods employed to detect degradation of lubricant in the early stages within an oil flow system can be segregated into continuous measurement or taking oil sample to laboratory for analysis. Any contamination or by-products present will lead to a change in dielectric constant. A method which can thus be used is to check the change in dielectric constant of the lubricant oil. Grid capacitance sensor is used, and the capacitance readout is precisely related to the input frequency. The following equation represents the correlation:

$C=1/2\ \pi f X_c$

Here, C represents the overall capacitance, whereas fis the input frequency and X_c is the inductive of component [31].

Spent and fresh oil were used to measure the dielectric constant and an empty shell was used as control. The variation range is from 6.5 to 10pF in relation to the frequency input. It was seen that the dielectric constant showed the maximum for used engine oil and empty shell (air) showed the least. In addition, three variables viz. ferrous particles, water contamination and SiO_2 particles were also taken into consideration [30]. Dielectric constant was measured between the three cause variables. The results obtained showed that ferrous particles and dust or water contamination have a rough output of 99% and the interaction among these three variables stand at an output of roughly 75%. A study of the plots of main and interaction effects gives us an idea of the considerable effect of water and dust contamination on sensor output. An extensive volume of work is required for assessment of factors such as metal particles, physical and chemical interactions. Spent lubricant oil from various stages of oil usage must be analyzed with the sensor, following which, it can be marketed.

A. CHEMICAL STRUCTURE AND LUBRICANT OIL STABILITY

There is a direct correlation between the chemical structure of oil imparting stability to the oil, and the biodegradability as well as toxicity of the lubricant. In literature, the hydrolytically stable base oils are categorized as a) excellent b) very good c) good d) moderate e) poor [21]. Considering example of a Rolls-Royce 1006 test [32, 33] it demonstrates how the stability of polyesters are affected by processing. Data is available on hydraulic oils which have been environmentally modified [34]. Using American Society for Testing and Materials (ASTM) D2619 procedure, the hydrolytic stability of base oils can be examined. It depends on the constituents such as hydrocarbons, esters and polyalkylene glycols present [35]. Certain parameters also play an important role. The parameters determined are TAN, hydrolytic, polyolester oils and temperature related stability [21]. Standard criteria need to be established for phosphate ester hydraulic fluids [36] and acceptable limits specified by pressure reducing valves have been stipulated [37].

IV. RHIZOSPHERE MICROFLORA IN LUBRICANT OIL BIODEGRADATION

While studying degradation effect of microbes on lubricant biodegradation occurs through small fraction of the dissolved compound during incubation period. This happens either by contact between cell and oil droplet contact or by oil phase solubilization [38].The effect of diesel and three types of lubricant oil was analyzed [39]. The study was performed on microbes in marine sediment in Antarctica. Different brands of were diesel used, viz. Special Antarctic Blend (SAB) and synthetic lubricant (Mobil 0W-40), a used form of the same lubricant and an unused equivalent Titan GT1. Using a ty6pe of gel electrophoresis, called DGGE (Denaturing Gradient Gel Electrophoresis), Most Probable Number (MPN) and Direct Microscope Count (DMC), the structure of microbial community was explored. Results showed minimum change in structure, but considerable differences in microbial community structure was observed after 5 weeks. It was seen that SAB and synthetic lubricants affected the sediment microbial communities to a greater extent. Considerable variation was not seen between control and unused biodegradable Titan GT1, which indicated that use of this type of oil could lower the hazard coupled with oil spills [39].

An argument can be presented in favor of phytoremediation over rhizoremediation. In phytoremediation, bacteria accumulate on the roots of the plants which already have indigenous bacterial population, and increase the bioremediation capacity [40] resulting in better soil quality, minimum harm to the surrounding environment and long-term beneficial effects. Oil spills in Huangpu-Yangtze River Estuary (HYRE) led to research on phytoremediation to solve the problem [41] and under the combined effect of plant and microbe a degradation rate in the range 28.28~52.94% was observed as opposed to only microbe degradation rate of 8.05~36.59%. But when phytoremediation is applied on a large-scale basis, many problems are encountered including limiting concentration of toxic elements which can be endured by the plants. Bioavailability is a very important factor when we talk about bioremediation. Also, contaminants are very often not bio-available and dissemination into the environment becomes a problem [42]. The answer can be found in usage of \contaminantt-specific, genetically engineered plants and also transforming the rhizosphere for boosting rhizodegradation [43, 44]. Studies have shown that various species of *Rhodococcus* and *Pseudomonas* identified by 16S rRNA gene sequencing, exhibit significant increase in number in plants grown in oil-contaminated soil [21]. Pure cultures of these bacteria were obtained, which demonstrated thriving growth on mineral media containing C9-C40 alkanes. Quantitative studies were also done which show degradation of crude oil to be about 20% -30%. Another feature of the cultures used was that they were nitrogen fixers, hence self-sufficient for nitrogen supply.

A.DEGRADATION OF LUBRICANT OIL BY A MIXED BACTERIAL CONSORTIUM

Biodegradation of lubricant oil in a bioreactor can be considered. This laboratory study was performed by using a biofilm carrier [45]. The consortium comprised of *Bacillus sp. Px2,Commaonas acidovoransPxl* and *Pseudomonas sp. Px3*. Lubricant oil degradation was from 99% to 97.2%. Total Dissolved Organic Carbon (TDOC) results showed that intermediates hardly accumulated. Scanning electron micrographsrevealed vigorous growth on bioreactor as biofilm which opens the benzene in initial stages [45]. Gradually all peaks radically reduced with increase in biomass and it was seen that n-alkane derivatives were better removed than branched alkane derivatives. This was supported by FT-IR analysis [46]. A combination of FT-IR spectroscopy and GC-MS and TDOC with removal of oil showed mixed consortia can break down benzene derivatives and aromatic organic compounds.

According to a study on car engine oil, both used and fresh oil were studied for biodeterioration potential of bacteria isolated from the engine [47]. The bacterial isolates were *Bacillus, Micrococcus, Corynebacterium, Serratia, Edwardsiella, Citrobacter, Pseudomonas,* and *Acinetobacter* species. Also, *Aspergillus, Cladosporium, Mucor, Cephalosporium, Penicillium* were the fungal genera isolated, along with *Saccharomyces*. Used oil was seen to provide a better substrate than unused oil, as one can predict. This is because used oil is already transformed into comparatively simpler compounds and easier for bacteria and fungi to utilise. These organisms were isolated from the car engine. Thus, studies can be done on such microbes from soil to enhance their degradation ability and use them abundantly for biodegradation of oil.

V. CONCLUSION

With the advent of revolution in use of vehicles worldwide, extensive research is being carried out to come up with environment friendly, biodegradable lubricants. The usage of lubricant oil cannot be lowered below a certain point, hence the best solution is to use microbial consortia or manipulate microbial communities in order to reduce the content of hydrocarbons from mineral oil-based lubricants in the soil. Moreover, the study of persistence of lube oil breakdown products in the environment holds urgency. Toxicity and persistence cannot be correlated in this case. The adverse effects, as well as the extent to which it remains in the environment, especially soil, need to be investigated simultaneously. Degradation mechanisms need to be worked out, which will present us with a better and clearer picture of the intermediates formed and insights on ways to improvise biodegradation methods.

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