



Biodegradation of Petroleum Gasoline and High Speed Diesel Oil

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Abstract

Fuel is the most dynamic chemical for growth of country as well human simultaneously it is the major pollutant among various dominant pollutant. Hazardous effect of pollutant over environment depends on intrinsic biodegradability of various compositional hydrocarbon of the fuel. Such a biodegradability of fuel like petroleum diesel and gasoline can be determine using some reference aerobic Microflora that can be achieved from waste water that crated by urban during domestic use of water. Among diesel and gasoline, Gasoline exhibited a high intrinsic biodegradability (96%) but that of a commercial diesel shows only 70%. The repeating hydrocarbons of fuels were cycloalkanes and branched alkanes, the composition in terms of various hydrocarbon structural classes responsible for the diverse biodegradation rates observed. In case of linear hydrocarbon like alkane, the biodegradation rate was found close to 100%. Capability of fuel biodegradation is depending on wide range of parameter that was tested here by using Microflorae. Among Microflorae of polluted soil and no polluted one from polluted soil having more efficiency for biodegradability. When Microflorae taken from polluted soil it proceed through various mechanism.

Keywords: Biodegradation; Petroleum; Diesel Oil

Introduction

Fuels are percolating in the ground state of soil due to of leaks of storage tanks and sometime defectiveness in transferring line of fuel. Such a stream of the fuels are proved crucial for the environment protection. There for it is important to do Biodegradation studies as it help us to understand in advanced the possible risks of fuel and also aid to define the most appropriate strategy to overcome this.

Various component of fuel released in the environment with the magnitude widely depends on the biodegradability of the component of hydrocarbons, using microflorae which is suitable for biodegradation and on various environmental factors. In this way, biodegradation studies are very much useful since they allow to identify the potential of microflorae for the natural degradation of fuel. At present, natural attenuation is regarded as a very efficient approach fro bioremediation. It is not used as proper cleanup alternative because it requires some action like site evaluation and contaminant migration towards sensitive dumping places such as wells or nearby situated lands. The estimation of the bioremediation potential of a polluted site is very much essential to decide natural attenuation which help to decide the cheapest remediation methodology. Several steps are useful to evaluate probability for

natural attenuation at a site. The first step related to the properties and characteristics of the pollutant is its intrinsic biodegradability. For biodegradation of some commercial compounds, microflorae having wide biodegradation range. The microflorae that use for such a purpose is taken from an activated sludge which accumulate during urban wastewater treatment. In these study, with the help of O₂ consumption or by CO₂ production biodegradation rate can be measured. By comparing this obtain data one can easily compared biodegradation rates of readily biodegradable products, e.g. glucose, or a n-alkane such as hexadecane in case of petroleum products degradation. While the second step is useful to know natural attenuation potential which indicates biodegradation capacities of Microflora. An interesting comparative study of the local microflora with a reference microflora, i.e. an activated sludge, In this way, several parameters must be taken into account: temperature, pH, nutrient and electron acceptor availability, water content of soil, transfer limitations in the soil matrix, in particular for dioxygen and pollutant.

The most water-insoluble components of gasoline, e.g. benzene, ethylbenzene, toluene, and xylene isomers, hydrocarbons usually termed as BTEX, have been clearly assessed using pure strains. Biodegradability of BTEX was checked using microflorae. Information

about biodegradation of most of gasoline component is not available except some straight chain alkane conjugated benzene and some branched alkane. Kinetic of biodegradation is further modified by interactions between individual strains. Many scientist have studied the degradation of petroleum diesel oils. Among various experiment biodegradation carried out by soil microcosms reported the incomplete degradation of petroleum diesel oil.

In current study, we will focused on biodegradability of gasoline and diesel oil for purpose to get enough information on identifiable hydrocarbons or structural hydrocarbon classes during biodegradation by using various microflorae from the environment.

Hydrocarbon nature of petroleum fuels

Hydrocarbon content of both the fuel diesel and gasoline is completely different. The number of carbon chain present in gasoline is in between 4 and 10. It has distillation profile is from 30-35°C to 180-200°C. While the number of carbon chain present in diesel is in between 11 and 25 and the distillation profile range is between 180 to 380°C.

Gasoline and diesel oil do not have any measure amounts of alkenes. They are both contained four main structural classes of hydrocarbons:

- n-alkanes (linear saturated hydrocarbons);
- Isoalkanes (branched saturated hydrocarbons);
- Cycloalkanes (saturated cyclic alkanes);
- Aromatics.

Gasoline having about 50% aromatic content of the total types of hydrocarbon content. Iso alkanes content is about 35%. And other class of hydrocarbon like Alkanes, alkenes and cycloalkanes are found in very minor proportion. There are total 230 different type of hydrocarbons are present in gasoline composition. Such a various type of hydrocarbons can be separated and identified by analytical instrument namely gas chromatography. While in diesel oil about 2000 to 4000 hydrocarbons, which are difficult to separate by gas chromatography. Although few n- alkanes and a few branched hydrocarbons can be identified as separated compounds. Separation of the main structural hydrocarbon classes can be carried out by using a standard procedure using analytical instrument namely high performance liquid chromatography.

Methodological approach for biodegradation studies

The biodegradability of unleaded gasoline and diesel oil can be done by test which is recommended by OECD (1993). The tests is performed with specific parameter, i.e. Temperature, Concerning

pH, Substrate concentration, Nutrient supply and oxygen availability. The tests is done in closed systems so one can do assessment of the carbon balance at the end of incubation period. The kinetics of biodegradation were measured for determining the end point of experiment. This testing was done on concept by either on di oxygen consumption by using instrument like electrolytic respirometry or carbon dioxide production testing by using gas chromatography. Once incubation period get finished, the residual hydrocarbons were quantified and the degradation rates were determined by comparing with hydrocarbons of abiotic control flasks in which microbial growth was inhibited by HgCl₂ addition.

Biodegradation of fuels by a microflora from an activated sludge

As previously indicated, the biodegradation characteristic of a commercial compound like gasoline by a microflora collected from urban waste water treatment plant, help us to know good initial approximation of its intrinsic biodegradability. Because of this, the activated sludge micro flora decided as a reference microflora.

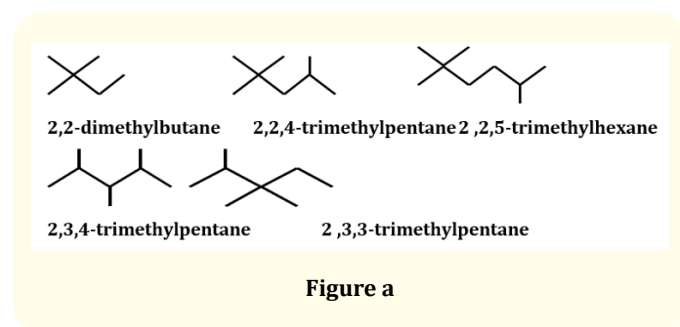
Hydrocarbon class	Degradation rate *(%)	Resistant fraction** (%)
n-alkanes	100	0
Methyl- and dimethylalkanes	98.3	0.5
Trimethylalkanes	51.3	2.8
Cycloalkanes	99.7	0
BTEX	99.9	0
Other aromatics	98.8	0.2
Alkenes	100	0
Total gasoline	96.5	3.5

Table 1: Biodegradation of gasoline by the reference microflora.

In above table we can see the data for degradation rates of the major hydrocarbon classes of gasoline by using the reference microflora from activated sludge. This microflora having tendency to degrade gasoline (up to 96%). All n- alkanes, like cycloalkanes, aromatics were totally consumed. The main repeating hydrocarbons belonged to the class of methylated alkanes. In specially, about 50% of initial trimethyl alkanes resisted to microbial degradation. Analytical study using instrument like Gas chromatography shown that the molecular recalcitrant structures were either branched hydrocarbons with a quaternary carbon or hydrocarbons with methyl groups on consecutive carbon atoms. As far concern to diesel oil degradation, the initial problem is the analytical complexity related to the huge number of components. As an example, the chro-

matographic separation profile of the commercial diesel oil used is shown in Figure. Such a profile displayed a enough satisfactory resolution for all n alkanes.

At the end of the incubation period, the profile of hydrocarbons showed that all n- alkanes were totally consumed by the reference microflora.



Initially procedure used for commercial diesel oil was implemented for measuring the degradation rates of various types of diesel oils that shown in following table. Result show that the commercial diesel oil was not entirely biodegradable. Furthermore, the response to biodegradation of other diesel oils was found depending on the molecular structure like in the case of gasoline. Hydrocracking diesel oil was found slightly less biodegradable than straight run diesel due to presence of the branched structures of the constituting hydrocarbons.

Diesel oil type	Degradation rate (%)	Mineralisation rate ** (%)
Commercial	73	62
Straight run	83	47
Hydrocracking	77	55
Fischer-Tropsch	98	60
Rapeseed methyl esters	100	79
Synthetic*	99	62
Commercial	73	62
Straight run	83	47

Table 2: Degradation of various types of diesel oil by the reference microflora.

Degradation capacities of microflorae from the environment

The gasoline degradation capacities of several microflorae of the environment were tested in comparison with those of the reference microflora. Two nonpolluted-soil microflorae and 6 polluted-soil microflorae were tested (Table 3).

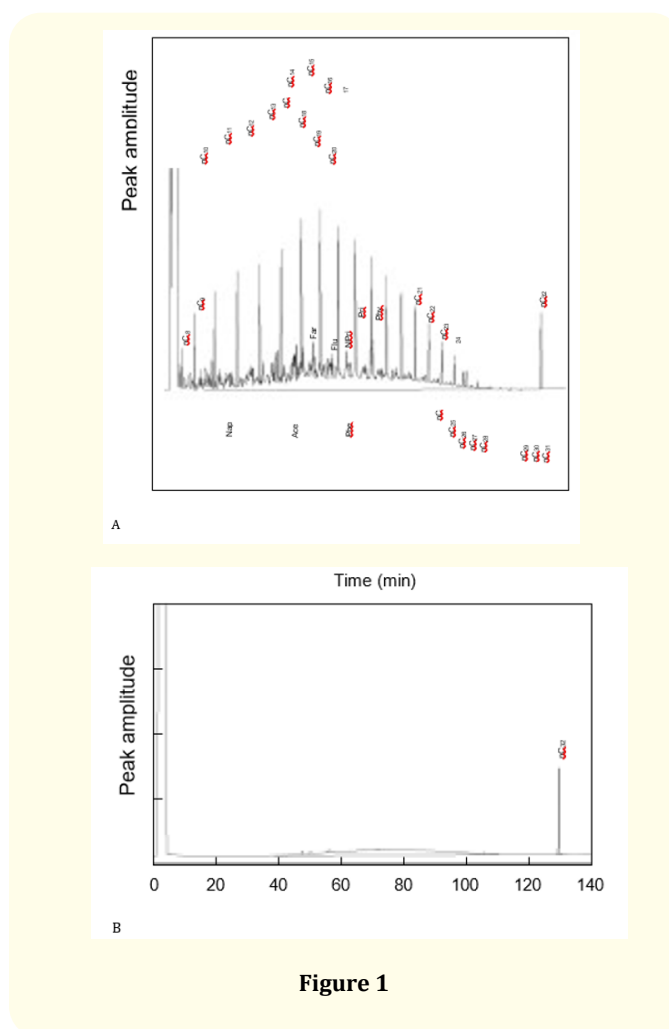


Figure 1

The hydrocarbon degradation rates observed with non polluted soils were found slightly lower side than with the reference microflora, in particularly, for cycloalkanes that displayed incomplete biodegradation. While the degradation rates measured with polluted soils were generally found higher than that of the reference microflora. Even it shows trimethyl alkanes were consumed by the polluted-soil microflorae. The degradation capacities of microflorae from the environment were similarly assayed on diesel oil (Table 4).

Two microflorae (microflorae 1 and 2) from different wastewater treatment plants shows different degradation capacities. The degradation characteristics of the refinery sludge where close to those of microflora 2 whereas those of the nonpolluted soil were found close to those of microflora 1. With the polluted soil, the level of biodegradation was higher than with the nonpolluted soil. In this case, a quite moderate mineralization rate was calculated. This rate seemed to indicate that intermediary metabolites could accumulate as a result of an incomplete degradation of the substrate.

Microflora	Degradation rate (%) for:					
	<i>n</i> -alkanes	Mono- and dimethylalkanes	Trimethylalkanes	Cycloalkanes	Aromatics	Total
Activated sludge	100	100	27	100	100	95
Spruce forest soil	100	92	14	29	98	89
Garden soil	99	100	28	75	100	94
Diesel oil-polluted soil	100	100	100	100	100	100
Jet fuel-polluted soil	100	96	17	85	85	85
Gasoline-polluted soil	100	100	100	100	100	100

Table 3: Gasoline degradation capacities of microflorae from the environment.

Microflora	Biodegradation rate* (%)	Mineralisation** (%)
Urban activated sludge 1	62	58
Urban activated sludge 2	73	63
Refinery activated sludge	75	68
Nonpolluted soil	65	58
Polluted soil	92	56

Table 4: Degradation capacities of various microflorae for commercial diesel oil.

Mechanisms of recalcitrant hydrocarbons degradation

The repetition of some hydrocarbon classes of fuels using non-polluted-soil microflorae showed the relation of microorganisms having specialized metabolic capacities in order to achieve a highest consumption of fuels. In polluted environments, the selective pressure resulting from pollution appeared to increase soils with bio degraders of cyclic or branched hydrocarbons. Actually, microflorae having specialty in degrading cycloalkanes or an isoalkane such as isooctane were obtained from polluted environments. Using an isooctane-degrading microflora, a pure strain was isolated. This strain and its specific properties quite well explained and exemplified the main mechanisms prevailing in the degradation of recalcitrant hydrocarbons in the environment. These mechanisms are the metabolism and strain cooperation.

The final balance of isooctane degradation is shown in (Table 5). The degradation data explain that more than 50% of the initial carbon was recovered in cell biomass plus CO₂. This clearly indicate that *M. austroafricanum* had the capacity to degrade the quaternary carbon atoms. Another interesting dynamicity of this specialized microorganism is the β -hydroxyacyl dehydrogenase this is because tertiary alcohols cannot be dehydrogenated. Since *M. austroafricanum* can grow on *anteiso*-methyl alkanes, it involved a metabolic pathway which can allowing β -oxidation to proceed. This pathway could involve a decarboxylase methylation step it has been already demonstrated in other microorganisms. Metabolism

ability of *M. austroafricanum* were demonstrated for cyclohexane degradation. *M. austroafricanum* was not able to utilize cyclohexane as a sole carbon source. However, when isooctane was treated and provided to the microorganism, cyclohexane was oxidized into cyclohexanone which accumulated. This *M. austroafricanum* could co metabolize cyclohexane when an energy substrate was available. A strain of *Acinetobacter lwoffii* was isolated for its capacity to degrade cyclohexane. It did not degrade cyclohexanone. When *M. austroafricanum* and *A. lwoffii* were cultivated simultaneously on cyclohexanone, cyclohexane was degraded but no cyclohexanone accumulated. Measurements of final CO₂ in the culture headspace showed that cyclohexane was totally mineralised. This illustrated the positive cooperative effect between the two strains for cyclohexane mineralisation. [1-26].

Conclusion

Using activated sludge Microflorae estimation about biodegradability of fuels can be carried out in flask tests. This test in case of gasoline gives very relative result with several microflorae. However, in the case of diesel oil, the relative study does not complies that much this is due to slightly different biodegradation rates of diesel. These variations is on account of differences in biodegradation capacities for branched alkanes which were present in large amounts in diesel oil compare to gasoline. Because of this gasoline was found highly biodegradable. About 97% of initial gasoline was degraded during the 27-day tests. In gasoline there were 96 major components, out of them 72 were completely degraded and remaining 28 were partially consumed. In case of diesel oil it is not much more biodegradable than gasoline. All simple straight chain *n*-alkanes and lighter hydrocarbons were degraded rapidly but some heavy branched hydrocarbons were not undergo biodegradation easily.

Degradation of recalcitrant structure hydrocarbons of fuel includes microorganisms having specific metabolic capacities. In polluted environments, specific microorganisms are found in profuse amount because of the adaptation of the microflorae to pollutant.

Working efficiency of this can be enhanced by two main mechanisms. The first one is the microorganism way by which a strain oxidize repetitive hydrocarbons when an energy source is available. The second one is just cooperation between strains. This two mechanisms explain why soil microflorae have extra ordinary capacities to eliminate pollution crated by fuel from contaminated environments.

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