

BIOREMEDIATION OF DIESEL AND BIODIESEL SOIL-CONTAMINATION BY USING MICROORGANISMS BIODEGRADABLE OIL

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Abstract: This paper address how the commercialization of biodiesel or the biodiesel/diesel blend can cause environmental damage due to spills during its extraction, transportation, processing, distribution and use urge the production of ecological fuel. When these oils containing polycyclic aromatic hydrocarbons (PAHs) spill into the soil, they penetrate into the deeper level of soil and causing ecological threat. The clean-up of these contaminated-areas can be achieved with method of bioremediation, a technique based on action of microorganisms, which turn hazardous contaminants into non toxic substances such as carbon dioxide (CO₂), water (H₂O) and biomass. This paper concludes on the needs for scientists and engineers to use an oil-biodegradable agent as an innovative treatment of oil-soil contamination through utilizes safe species of bacteria and not produces adverse effects to the human health or the environment. The advances technology platform is highlighted with multi disciplinary approach towards providing innovative and sustainable solution to oil-soil contamination problems.

Keywords: Biodiesel, diesel, soil-oil contamination, microorganism biodegradable oil

1. Introduction

Biodiesel and diesel have very distinct origins and compositions. Biodiesel is composed of methyl or ethyl esters of fatty acids with low structural complexity like oleate, palmitate, estearate, linoleate, myristate, laureate and linolenate derived from different vegetable oil sources such as soybean, sun-flower, peanut, cotton, palm oil, coconut, babassu and castor oil and from animal fat [1]. Similar properties of the diesel fuel and biodiesel as well as the requirements of the fuel strategy of the European Union (Biofuels Directive 2003/30/EC) have resulted in the emergence of mixtures of both biodiesel and diesel fuel on the markets of many countries; however this means new threats to the environment [2]. Even though biodiesel is considered to be easily biodegradable, it is a foreign compound in natural environment, as conventional diesel fuel.

The commercialization of biodiesel or the biodiesel/diesel blend can cause environmental damages due to spills. Oil contaminated soil is unsuitable for agricultural, residential or recreational uses that have become a major environmental issues in many industrialized countries. In addition, soil pollution may cause enormous economic loss, ecological disaster and destroy agricultural production [3]. The clean-up of these contaminated areas can be achieved with method of bioremediation, a technique based on the action of microorganisms, which turn hazardous contaminants into

non-toxic substances such as CO₂, water and biomass [4]. This technique is also an option to destroy or render harmless various contaminants using natural biological activities. In this sense, engineered bioremediation is a remediation technique based on strategies that accelerate the biological breakdown of the pollutant by stimulation of the indigenous microorganisms. *Pseudomonas* demonstrates a very diverse metabolism, including the ability to degrade organic solvents such as toluene [5]. This ability has been put to use in bioremediation, or the use of microorganisms to biodegrade oil. The use of *P. putida* is preferable to some other *Pseudomonas* species capable of such degradation because it is a safe species of bacteria, unlike *P. aeruginosa* for instance, which is an opportunistic human pathogen [5].

Thus, the assessment of the capability of *P. putida* will be studied to accelerate the biodegradation of biodiesel and diesel.

2. History of biodiesel

The history of biodiesel began in the 1880s, when Rudolf Diesel designed a compression engine using peanut oil. He desired to show that this was a far better method than the use of fossil fuels [6]. Dr. Rudolf Diesel, a son of Bavarian immigrants to France who become a machinist, a designer, and a noted refrigerator engineer invented the first "Diesel" engine in 1892 [7]. In fact, he

designed it to run on a number of fuels, including vegetable oil and then he successfully demonstrated his engine at the World Exhibition in Paris in 1900 and described an experiment using peanut oil as fuel in his engine. In 1912, R. Diesel said, "The use of vegetable oils for engine fuels may seem insignificant today. But such oils may become in course of time as important as petroleum and the coal tar products of the present time" [8].

2.1 Biodiesel

Biodiesel is a fuel composed of mono-alkyl esters of long chain fatty acids, derived from a variety of vegetable oils or animals fats. It is known chemically as a "methyl ester". In simpler term, biodiesel is the end product of a vegetable oil (triglycerides) or animal fats that has been chemically react with an alcohol and a catalyst to remove the glycerine, the chemical produced is known as fatty-acid methyl ester or biodiesel that attached to glycerol. To achieve this reaction, a strong "catalyst" such as Sodium Hydroxide (NAOH) or Potassium Hydroxide (KOH) must be added to the alcohol [7].

Biodiesel can be made from methanol or from ethanol which can be derived from crops such as corn. Other "higher" alcohols such as isopropanol and butanol have also been used, but there are numerous problems with using them. Using alcohols oh higher molecular weights improves the cold flow properties of the resulting ester, at the cost of a less efficient transesterification. When made from methanol the ester is called methyl ester, and when made from ethanol it's called ethyl ester [7].

i. Biodiesel production:

There are four primary methods to make biodiesel such as direct use and blend, micro emulsions, thermal cracking (pyrolysis) and transesterification. The most commonly used method is transesterification of vegetable oils and animal fats [7]. The transesterification reaction is affected by molar ratio of alcohol, presence of water and free fatty acid content, reaction temperature, catalyst concentration and agitation speed [9].

ii. Transesterification process:

Biodiesel is commonly synthesis through a process known as transesterification (alcoholysis) which involves a reaction between a short-chain alcohol and glyceride containing oil (can be plant oil, vegetable oil, fat or grease) [6]. The purpose of the process is to lower the viscosity of oil. A catalyst is usually used to improve the reaction and yield. Because the reaction is reversible, excess alcohol is used to shift the equilibrium to the product site. [10]. The chemical reaction of biodiesel is shown in **Fig 2.1**.

Triacylglycerol (triglycerides), as the main component of vegetable oils and animals fats, consist of

fatty acids with three long chains esterified to a glycerol back bone [12]. R₁, R₂ and R₃ indicate the fatty acid

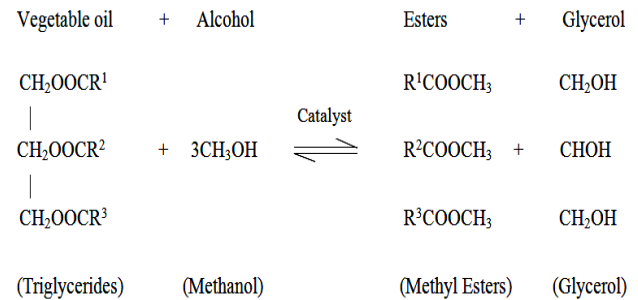


Fig 2.1: Transesterification of triglycerides with alcohol [11]

chains associated with the oil or fat, which are largely palmitic, stearic, oleic, or linoleic in nature. Methanol and ethanol are the most commonly used alcohol because of the low cost. Supercritical methanol has a high potential for both transesterification of triglycerides and methyl esterification of free fatty acids to methyl ester for a diesel fuel substitute [11]. Next, both homogenous catalyst and heterogeneous catalyst are used in biodiesel production. The homogenous catalysts are alkalis such as NAOH or KOH [13] and acid catalyst such as Sulfuric Acid (H₂SO₄) or Hydrochloric Acid (HCL) [14]. The heterogeneous catalyst includes sugar catalyst [15].

2.2 The material used in biodiesel production

2.2.1 Vegetable Oil

There are numerous vegetable oils derived from various sources. These include the popular vegetable oil: the foremost oilseed oils-soybean, cotton seed, pea-nuts and sunflower oils; and other such as palm oil, palm kernel oil, coconut oil, castor oil, rapeseed oil and others. Their yields, different compositions and by extension their physical and chemical properties determine their usefulness in various application aside edible use [16].

The oil palm produces two types of oils, palm oil from the fibrous mesocarp and lauric oil from the palm kernel. In the conventional milling process, the fresh fruit bunches are sterilized and stripped of the fruitlets which are then digested and pressed to extract the crude palm oil (CPO). The nuts are separated from fibre in the press cake and cracked to obtain palm kernels which are crushed in another plant to obtain palm kernel oil (PKO) and by-product, palm kernel cake which is used as an animal feed. Fractionation of CPO and PKO in the refinery produces the liquid stearin fraction and a solid stearin component [17].

Palm oil is mixes of fatty acids, especially three-unsaturated, result lower viscosity, but more susceptible to oxidation. In the other side, high percentage of saturated fatty acids oils result higher resistance to oxidation, but

have a higher viscosity and pour point. The best choice is monounsaturated [18].

By searching on palm oil and it's by product, it is found that Refined, Bleach and Deodorized Palm Oil (RBDPO) Olein has a highest content of monounsaturated fatty acids, especially oleic. **Table 2.1** shows fatty acids content of palm oil and it's by product. The oils are CPO, PKO, RBDPO, RBDPO Olein, RBDPO Stearin, and Palm Fatty Acid Distillate (PEAD).

Table 2.1: Fatty acids content of palm oil and it's by product ^[19]

Fatty Acid	Percentage (weight)				
	CPO	PKO	RBD Oleum	RBD Stearum	PEAD
Lauric (12:0)	0.2	47-53	0.1-0.5	0.1-0.6	0.1-0.3
Miristic (14:0)	1.1	15-19	0.9-1.4	1.1-1.9	0.9-1.5
Palmitic (16:0)	44	8-11	37.9-41.7	47.2-73.8	42.9-51.0
Stearic (18:0)	4.5	1-3	4.0-4.8	4.4-5.6	4.1-4.9
Palmatoleic (16:1)	-	-	0.1-0.4	0.05-0.2	-
Oleic (18:1)	39.2	12-19	40.7-43.9	15.6-37.0	32.8-39.8
Linoleic (18:2)	10.1	2-4	10.4-13.4	3.2-9.8	8.6-11.3

Palmatoleic (16:1) mean its fatty acid chain containing 16 carbons atom, one of carbon-carbon bond is a double bond. The same way, Olein (18:1) meaning the fatty acid chain consists of 18 carbons atom, one of carbon-carbon bond is double bond. Therefore, Oleic and Palmatoleic are monounsaturated fatty acids. Actually, the fatty acids belong to the class of aliphatic acids, such as Palmitic (16: 0), Stearic (18:0) and Oleic (18:1) in animal and vegetable fats and oils. The major fatty acids in palm oils are Myristic (14:0), Palmitic (16:0), Stearic (18:0), Oleic (18:1) and Linoleic (18:2) [20]. The typical fatty acid composition of palm oil from Malaysia is presented in **Table 2.2**.

2.2.2 Catalyst

Transesterification reactions can occur in the absence of catalyst but it requires high temperature, pressure and long reaction times. Three types of catalyst are used in the production of biodiesel such as basic catalyst, acid catalyst, and biocatalyst [21].

a. Basic Catalyst

Alkali catalyzed transesterification is much faster than other type of the catalyst. Alkalis include sodium hydroxide (NAOH), sodium hydride (NAH), potassium hydroxide (KOH) and potassium hydride (KH) [22].

Alkaline metal hydroxides like KOH and NAOH are preferred as the basic catalyst. However, in the alkaline metal hydroxide-catalyzed transesterification, even if a water free vegetable oil and alcohol are used, certain amount water is produced from the reaction of the hydroxide with alcohol [23]. The presence of water leads to the hydrolysis of the esters, and as a result, soap is formed (**Fig 2.2**). The formation of soap reduces the

Table 2.2: The typical fatty acid composition of palm oil from Malaysia ^[20]

Fatty acid chain length	Mean	Range observed	Standard deviation
12: 0	0.3	0-1	0.12
14: 0	1.1	0.9-1.5	0.08
16: 0	43.5	39.2-45.8	0.95
16: 1	0.2	0-0.4	0.05
18: 0	4.3	3.7-5.1	0.18
18: 1 (n-9)	39.8	37.4-44.1	0.94
18 : 2 (n-6)	10.2	8.7-12.5	0.56
18 : 3	0.3	0-0.6	0.07
20 : 0	0.2	0-0.4	0.16

biodiesel yield, and causes significant difficulty in product separation (ester and glycerol). Therefore, for alkali-catalyzed transesterifications, the triglyceride must contain low levels of the free fatty acids (FFAs) and the alcohol used is essentially anhydrous to reduce the formation of unwanted soap.

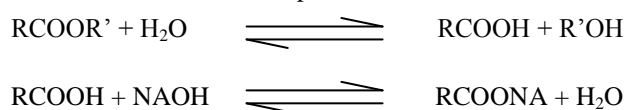


Fig 2.2: Saponification of fatty acid alkyl ester ^[23]

b. Acidic Catalyst

If glyceride has a higher level of FFA and more water then acid catalyzed transesterification suitable, that can be sulphuric (H₂SO₄), phosphoric, hydrochloric (HCL) or organic sulfonic acid. Acid catalyzed methylation is energy sensitive because it is usually conducted at high temperature. Palm oil with H₂SO₄ gives highest yield of 78% of methyl esters at 23:1 ratio [24].

Acid catalyst such as HCL and H₂SO₄ give very high yields of alkyl esters but the reactions are slow and require temperature above 100 °C for longer reaction time [23]. The only advantage of this type of catalytic conversion is that it can efficiently esterifies free fatty acids in vegetable oils and is therefore used to

To ensure that the manufactures have removed excess methanol used during the production of methyl ester, the flash point is set above 120 °C 130 °C in the respective EN14214 and ASTM D6571. Residual methanol is a safety issue as even a very small amount will reduce the

flash point greatly and it can also affect fuel pumps, seals and elastomers. Actually, the properties such as the methanol, monoglycerides, diglycerides and triglycerides indicate the degree of conversion from oil to methyl esters or completion of the transesterification process [34].

Table 2.3: European Standard for Biodiesel (EN 14214) [34]

Property	Unit	Grade S15	Grade S500	Test Method
		Limits	Limits	
Kinematic viscosity at 40 °C	mm ² s ⁻¹	1.9-6.0	1.9-6.0	ASTM D445
Flash point (closed up)	°C	130.0 min	130.0 min	ASTM D93
Sulphur content	% mass (ppm)	0.0015 max (15)	0.05 max (500)	ASTM D5453
Carbon residue (on 100% distillation residue)	% mass	0.050 max	0.050 max	ASTM D4530
Acid number	mg KOH g ⁻¹	0.80 max	0.80 max	ASTM D664
Cloud point	°C	Report*	Report*	ASTM D2500
Cetane number	-	47 min	47 min	ASTM D613
Sulphated ash content	% mass	0.020 max	0.020 max	ASTM D874
Water and sediment	% volume	0.050 max	0.050 max	ASTM D1796
Copper strip corrosion (3 hr at 50 °C)	rating	N0. 3 max	N0. 3 max	ASTM D130
Free glycerol	% mass	0.020	0.020	ASTM D6584
Total glycerol	% mass	0.240	0.240	ASTM D6584
Phosphorus	% mass	0.001 max	0.001 max	ASTM D4951
Distillation temperature (90% recovered)	°C	360 max	360 max	ASTM D1160
Note: * The cloud point of biodiesel is generally higher than that of petroleum-based diesel and should be taken into consideration when blending.				
Source: ASTM International (2003)				

Table 2.4: Standard Specification for Biodiesel Fuel (B100)

Property	Unit	Limits		Test method
		Minimum	Maximum	
Ester content	% (mm ⁻¹)	96.5	-	pr EN 14103
Density at 15 °C	kgm ⁻³	860	900	EN ISO 3675
Viscosity at 40 °C	mm ² s ⁻¹	3.5	5.0	EN ISO 12185
Flash point	°C	120	-	ISO/CD 3679
Carbon residue (on 10% distillation residue)	% (mm ⁻¹)	-	0.3	EN ISO 10370
Acid value	mg KOH g ⁻¹	-	0.5	pr EN 14104
Cetane index	-	51.0	-	EN ISO 5165
Sulphur content	mg kg ⁻¹	-	10	-
Sulphated ash content	% (mm-1)	-	0.02	ISO 3987
Water content	mg kg ⁻¹	-	500	EN ISO 12937
Total contamination	mg kg ⁻¹	-	24	EN 12662
Copper strip corrosion (3hr at 50 °C)	Rating	1	-	EN ISO 2160
Oxidation stability, 110 °C	hr	6.0	-	pr EN 14112
Iodin value	-	-	120	pr EN 14111
Linolenic acid methyl ester	% (mm ⁻¹)	-	12	pr EN 14103
Polyunsaturated (> 4 double bonds) methyl esters	% (mm ⁻¹)	-	1	-
Methanol content	% (mm ⁻¹)	-	0.2	pr EN 14110
Monoglyceride content	% (mm ⁻¹)	-	0.8	pr EN 14105
Diglyceride content	% (mm ⁻¹)	-	0.2	pr EN 14105
Triglyceride content	% (mm ⁻¹)	-	0.2	pr EN 14105
Free glycerol	% (mm ⁻¹)	-	0.02	pr EN 14105
				pr EN 14106
Total glycerol	% (mm ⁻¹)	-	0.25	pr EN 14105
Alkaline content (Na + K)	mg kg ⁻¹	-	5	pr EN 14108
				pr EN 14109
Phosphorus content	mg kg ⁻¹	-	10	pr EN 14107

Source: European Committee for Standardization (CEN) (2003)

Biodiesel is miscible with petrodiesel in any proportion and possess several technical advantages over ultra-low sulfur diesel fuel (ULSD, <15 ppms), such as inherent lubricity, low toxicity, derivation from a renewable and domestic feedstock, superior flash point and biodegradability, negligible sulfur content, and lower overall exhaust emissions [35]. Sulfur oxides produced from combustion of the fuel react with moisture present in the combustion chamber to form sulphuric acid which corrodes the cylinder liner and piston. Besides a negative effect on diesel engines, the sulfur content in fuel is also a concern towards the environment as it contributes to air pollution by forming acid rain.

An upper limit of 0.02% sulphated ash was set in both standards to ensure total removal of the catalyst used in the production. High sulphated ash may cause deposits and filter plugging while high acid number is normally associated with fuel system deposits on pumps and filter. So, the palm diesels have sulphated as contents of not more than 0.01%.

2.3.2 Fuel properties of process liquid palm oil/ petroleum diesel blend based on Malaysian Standard on diesel fuel

Malaysian Palm Oil Board (MPOB) palm biofuel programmed has used processed liquid palm oil (PLPO) and its blend with petroleum diesel (PD). The results shown that the blend of PLPO/PD (up to 10% PLPO) can be used directly modification in conventional diesel engines after the evaluation of the physical and fuel

properties of these palm biofuels samples in various ratios.

Biodiesel can be blended and used in many different concentration. They include B100 (pure biodiesel), B5 (5% biodiesel, 95% petroleum diesel), and B2 (2% biodiesel, 98% petroleum diesel). The most common biodiesel blend is B20, which qualifies for fleet compliance under Energy Policy Act (EPA) of 1992 [36]. Since 2002, actual engine trials using MPOB vehicle on B2, B5 and B10 have been carried out. So far, not technical problems have been reported and the long term effects on the engines are being studied.

There is currently no biodiesel standard for vegetable oil/diesel blends since the global development of biodiesel is more focused on the methyl esters of vegetable oil. Both the European and American Standards on Biodiesel are concentrated for methyl esters and, in the respect, both standards are not suitable for evaluating of PLPO/PD blends (B2, B5, B10). Therefore, it is appropriate to use the Malaysian Standard for Diesel Fuel (MS123:1993) as the reference standard.

Generally, the fuel properties of PLPO/PD blends are very similar to those of petroleum diesel. Blending PLPO up to 10% in petroleum diesel does not change the fuel properties of petroleum diesel [34]. **Table 2.6** depicts the fuel properties of the blends from a study conducted using PLPO from different palm oil refineries in Malaysia.

Table 2.5: Fuel Properties of Normal and Low Pour Point Palm Diesel ^[34]

Property	Unit	Normal palm diesel	Low pour point palm diesel	EN14214	ASTM D6751
Ester content	% mass	98.5	98.0 to 99.5	96.5 (min.)	-
Density at 15 °C	kg litre ⁻¹	0.8783	0.87 to 0.89	0.86 to 0.90	-
Viscosity at 40 °C	mm ² s ⁻¹	4.415	4 to 5	3.5 to 5.0	1.9 to 6.0
Flash point	°C	182	150 to 200	120 (min.)	130 (min.)
Cloud point	°C	15.2	-18 to 0	-	Report
Pour point	°C	15	-21 to 0	-	-
Cold filter plugging point	°C	15	-18 to 3	-	-
Sulphur content	% mass	<0.001	<0.001	0.001 (max.)	0.0015 (min.) (Grade S15) 0.05 (min.) (Grade S500)
Carbon residue (on 10% distillation residue)	% mass	0.02	0.02 to 0.03	0.3 (max.)	0.05 (max.)
Acid value	mg KOH g ⁻¹	0.08	<0.3	0.5 (max.)	0.8 (max.)
Sulphated ash content	% mass	<0.01	<0.01	0.02 (max.)	0.02 (max.)
Basic sediment and water	% mass	<0.05	<0.05	0.05 (max.)	0.05 (max.)
Cetane number	-	58.3	53.0-59.0	51 (min.)	347 (min.)
Copper strip corrosion (3 hr at 50 °C)	rating	1a	1a	1	3 (max.)
Iodine value	-	52	56 to 83	120 (max.)	-
Content of linolenic acid methyl esters	% mass	<0.5	<0.5	12 (max)	-
Content of polyunsaturated fatty acid methyl esters (more than 3 double bonds)	% mass	<0.1	<0.1	1 (max.)	-
Methanol content	% mass	<0.2	<0.2	0.2 (max.)	-
Monoglycerides content	% mass	<0.4	<0.4	0.8 (max)	-
Diglycerides content	% mass	<0.2	<0.2	0.2 (max.)	-
Triglycerides content	% mass	<0.1	<0.1	0.2 (max.)	-
Free glycerol content	% mass	<0.01	<0.01	0.02 (max.)	0.02 (max)
Total glycerol content	% mass	<0.01	<0.01	0.25 (max)	0.24 (max.)

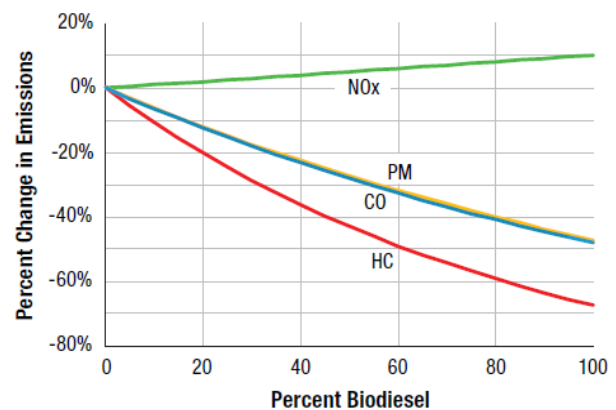
Notes: ASTM D6751 : Standard Specifications for Biodiesel Fuel (B100) Blend Stock for Distillate Fuels
EN14214 : European Standard for Biodiesel

Table 2.6: Fuel Properties of PLPO/ Malaysian PD Blend. ^[34]

Property	Unit	PLPO/PD blends		Malaysian petroleum diesel	MS123: 1993	
		B2	B5		Min.	Max.
Density at 15°C	kg litre ⁻¹	0.8395 - 0.8448	0.8419 - 0.8459	0.83 - 0.842	-	-
Viscosity at 40 °C	mm ² s ⁻¹	3.974 - .184	4.136 - 4.549	3.918 - 3.974	1.5	5.8
Flash point	°C	77.0 - 81.0	75.0 - 81.0	77.0	60	-
Cloud point	°C	14 - 16	14 - 16	14 - 16	-	18
Pour point	°C	9 - 12	9 - 12	12	-	15
Sulphur content	mg kg ⁻¹	0.18 - 0.19	0.17 - 0.18	0.19 - 0.21	-	0.5
Carbon residue (on 10% distillation residue)	% mass	< 0.1 - 0.1	0.2	< 0.1	-	0.2
Cetane number	-	50.6 - 61.8	54.8 - 61.5	55.2 - 61.2	45	-
Cetane index	-	51 - 57	55 - 56	51 - 57	47	-
Ash content	% mass	0.001 - 0.007	<0.001 - 0.006	0.001 - 0.005	-	0.1
Basic sediment and water	mg kg ⁻¹	< 0.01	< 0.01	< 0.01	-	0.1
Copper strip corrosion (3 hr at 100 °C)	rating	1a	1a	1a	-	1
Distillation temperature at 90% recovery	°C	365.4 - 369.0	363.7 - 367.8	365.4 - 365.9	-	370
Water by distillation	% volume	< 0.05	< 0.05	< 0.05	-	0.05
Colour	-	1.0 - L 1.5	1.0	L1.0 - 1.5	-	2.5
Gross calorific value	MJ kg ⁻¹	45 - 47	44 - 47	45 - 47	-	-
Notes						
B2: 2% processed liquid palm oil + 98% petroleum diesel.						
B5: 5% processed liquid palm oil + 95% petroleum diesel						
MS123: 1993: Malaysian Standard for Diesel Fuel						

2.4 Benefits of Biodiesel

The use of biodiesel in conventional diesel engines substantially reduces emissions of unburned hydrocarbons (HC), carbon monoxide (CO), sulfates polycyclic aromatic HCs, nitrated polycyclic aromatic HCs, and particulate matter (PM). As the amount of biodiesel blended into diesel fuel increase will result in the enhancement of the reduction of the compounds [36]. B100 provides the best emissions reduction, but lower-level blends also provide benefits. B20 has been shown to reduce PM emissions by 10%, CO by 11%, and unburned HCs by 21% (**Fig 2.4**). Studies of oxides of nitrogen emissions have provided contradictory results, and additional testing and analysis is ongoing [36]. Biodiesel use also reduces greenhouse gas emissions because the CO₂ released in biodiesel released in biodiesel combustion is offset by the CO₂ sequestered while growing the feedstock. B100 use reduces CO₂ emissions by more than 75% compare to diesel and then using B20 reduces CO₂ emissions by 15%



Source: EPA, Draft Report EPA 420-P-02-001, October 2002

Fig 2.4: Average Emission Impacts of Biodiesel for Heavy-Duty Highway Engines. ^[36]

There are many distinct benefits of using biodiesel compare to diesel fuel [7]:

- i. It is made from renewable resources and reduces our dependence on oil from foreign countries and contributes to our own society.
- ii. It's plant-based and considered carbon neutral, because it adds almost no CO₂ to the atmosphere, since the plants that it's made from consume of CO₂ and produce oxygen (O₂).
- iii. It can be used straight in warmer weather (as B100) or can be blended with petrodiesel in any ration and splash mixes with no mechanical mixer need.
- iv. Toxicity test show that biodiesel is considerably less than diesel fuel.

2.5 Diesel Fuel

Diesel, petrol and other crude oil distillation products are complex chemical mixtures, containing hundreds of chemical substances, which can be mainly divided into aliphatic and aromatic hydrocarbons with small amounts of additives [37]. Petroleum products contain varying percentages of hydrocarbon, depending on the distillation process used to generate them, and alkylated and non-alkylated aromatic hydrocarbons containing two or three aromatic rings are generally present in aqueous phases polluted by these products [38]. Polycyclic aromatic hydrocarbon (PAHs; two or more rings) raise substantial concern because of their widely known toxic potential, including mutagenic, ferotegenic, carcinogenic, photo-induced toxicity and endocrine-disrupting activities [39].

Accidental release of petroleum products from pipelenes and storage tanks are among the most common cause of soil contamination [40]. Petroleum hydrocarbon pollution is the one of the main environmental problems, not only by the significant amounts released but also by their toxicity. Among those petroleum hydrocarbon, fuel oil and diesel are more difficult to treat compared to gasoline due to their characteristics of lower volatility, lower biodegradability, higher viscosity, and lower mobility [41].

Among several clean-up techniques available to remove petroleum hydrocarbons from the soil on groundwater, bioremediation process is gaining ground due to their simplicity, higher efficiency and cost-effectiveness when compared to other technologies [42]. These processes rely on the natural ability of microorganisms to carry out the mineralization of organic chemicals, leading ultimately to the formation of CO₂, water (H₂O) and biomass [43].

2.5.1 Refining Process

Crude oil in its natural state has no value to consumers and must be transformed into products that can be used in the market place. Various physical and chemical methods are used in refining processes. Heat, pressure, catalysts, and chemicals are applied under

widely varying process designs, operating conditions, and chemicals reactions to convert crude oil and other hydrocarbons into petroleum products [54]. Petroleum refining process and operations (**Fig 2.5**) are classified into five basics types [44]:

- i. Distillation is the separation of crude oil in atmospheric and vacuum distillation columns into groups of hydrocarbon compounds based on molecular size and boiling-point ranges.
- ii. Conversion process change the size or structure of hydrocarbon molecules by;
 - Decomposition:** Breaking down large molecules into smaller molecules with lower boiling points through cracking and related processes.
 - Unification:** Building small molecules into larger molecules through alkylation, polymerization, and related processes.
 - Reforming:** Rearranging molecules into different geometric structures in insomerization, catalytic reforming, and related processes.
- iii. Treatment processes prepare hydrocarbon streams for additional processing and to prepare finished products using chemical or physical separation. Processes include desalting hydrodesulfurization, solvent refining, sweetening, solvent extraction and dewaxing.
- iv. Blending is the process of mixing and combining hydrocarbon fractions additives and other components to produce finished products with specific performance properties.

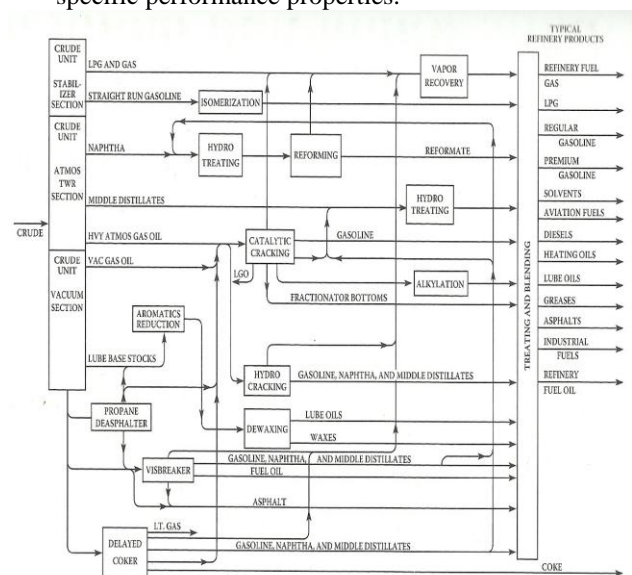


Fig 2.5: Refinery flow diagram. [44]

2.5.2 Petroleum Hydrocarbons

Petroleum hydrocarbons refer to the mixture of compounds in petroleum product that are all made mainly from hydrogen and carbon, hence “hydrocarbon”. These compounds can be categorized into four simple fractions [45]

- i. Saturates (or alkanes).
- ii. Aromatics, including such compounds as benzene, toluene, ethylbenzene and xylenes (BTEX) and polyaromatic hydrocarbons (PAHs).
- iii. Resins, consisting of compounds containing nitrogen, sulfur, and oxygen that are dissolved in oil.
- iv. Asphaltenes, which are large and complex molecules that are colloidally dispersed in oil.

The relative proportions of these fractions of these fractions are dependent on many factors such as source, age and migration. Of these fractions, the shorter alkane chain compounds and the lighter aromatics such as BTEX tend to be more readily biodegradable [45].

There are several techniques that can be applied to enhance the biological degradation of petroleum hydrocarbon contaminants and speed up the restoration of soil and groundwater [45]:

- i. Supplementation with suitable sources of nitrogen and phosphorus to enhance biodegradation of site contaminants by indigenous microbial population.
- ii. Enhancing the oxygen concentration by injection or infusion of air, oxygen, or slow oxygen release compound (ORCs) to optimize aerobic biodegradation of petroleum hydrocarbon contaminants.
- iii. Applying surfactants to enhance the bioavailability of the hydrocarbon contamination.
- iv. Bioaugmentation. If the indigenous microbial population is low or inadequate (for instance, due to toxicity), key microorganisms can be isolated from the site, grown up in large volumes, and used for inoculation. However, this is rarely required for the treatment of petroleum hydrocarbons.

2.6 Soil

Soil health is defined as the continued capacity of soil to function as a vital living system, by recognizing that it contain biological elements that are key to ecosystem function within land-use boundaries [46]. The functions are able to sustain biological productivity of soils, maintain the quality of surrounding air and water environments, as well as promote plant, animals, and human health [47].

Soil quality was defined as “the capacity of reference soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation [46]. The soil quality can also be defined as the capacity of a soil to function within the ecosystem boundaries and to interact positively with surrounding ecosystem [48].

In Asia, adverse effect on soil health and soil quality arise from nutrient imbalance in soil, excessive fertilization, soil pollution, and soil losses processes [49] [50]. Due to these problems, soil quality began to be interpreted as a sensitive a dynamic way to document soil

condition, response to management, or resistance to stress imposed by natural force or human uses [51].

2.6.1 Basic Soil Properties

2.6.1.2 Soil Physical Properties

Soil physical properties can be estimated from the soil’s texture, bulk density, and porosity [52]. The presence or absence of hard pans usually presents barriers to rooting depth. These properties are all improved through additions of organic matter to soils. Therefore, the suitability of soils for sustaining plant growth and biological activity is a function of its physical properties.

a. Soil Texture

There are 12 soil textural classes that are defined by the relative proportion of sand, silt and clay that makes ups a soil sample (Figure 2.6). There are two generally used methods for determining soil texture: The field method done by hand and mechanical analysis.

Using the mechanical analysis methods to determine the sand, silt, and clay requires the removal of course fragments by sieving the soil through a 2-mm sieve. Chemical treatments are also necessary to remove cementing agents such as organic matter and carbonates. The percent sand, silt, and clay should always total 100%, and once known, the soil texture can be found by using the textural triangle (Figure 2.6).

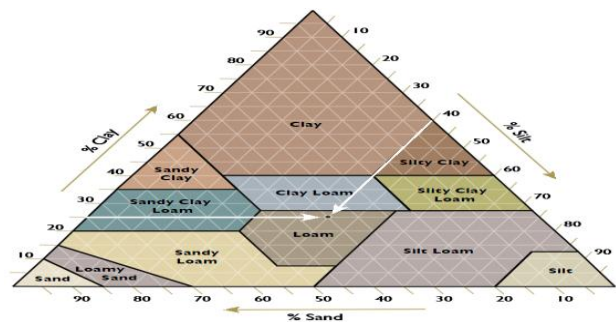


Figure 2.6: Textural triangle showing a soil’s textural class according to the percentage of sand, silt and clay it contains. [53]

Soil texture is often consider a basic property of the soil because the textural class of a particular soil generally remains unchanged over time equivalent to the human lifespan. This is one reason soil descriptions used in soil surveys records the soil textural class of each horizon. However, a disturbance of an area, such as water and wind erosion, could alter the textural class of the soil surface of both the soil being eroded and the soil where the erosional deposition occurs. Over long periods (geologic time), weathering and translocation of soil materials may change soil texture [53].

b. Bulk Density

A soil's bulk density relates to the volume of mass of dry soil plus its air spaces. It is measures of the mass per volume (Mg/m³). Soils with high bulk density are less porous and less permeable. For instance, clay soils have high bulk density. Their particles are tightly packed with minimal air spaces between them. Clays that swell when they become wet, seal off soil pores further, and can virtually stop the flow of water. Approximately half of the volume of the soil consists of different-sized pore spaces. When water enters the soil, it displaces air. Soils with high densities have slow water infiltration and permeability, which can result in pounding and run off [54].

c. Soil Porosity

Many important soil processes take place in pores (the air or water-filled spaces between particles). Soil texture and structure influence porosity by determining the size, number and interconnection of pores. Coarse-textured soils may have more small pores. Macropores in fine-textured soils exist between aggregates. Because fine-textured soils have both macro and micropores, they generally have a greater total porosity, or sum of all pores, than coarse-textured soils [55].

Unlike texture, porosity and structure are not constant and can be altered by management, water and chemical processes. Long term cultivation tends to lower total porosity because of decrease in soil organic matter (SOM) and large peds [56]. Surface crusting and compaction decrease porosity and inhibit water entry into the soil, possibly increasing surface runoff and erosion. Calcareous and salt-affected soils can also alter porosity and structure. In general, increasing SOM levels, reducing the extent of soil disturbance, and minimizing compaction and erosion will increase soil porosity and improve structure [55].

2.6.1.2 Soil Chemical Properties

a. Soil organic matter

Organic matter plays an important role in the chemistry of soils. Soil properties associated with soil organic matter include soil structure, macro- and micronutrient supply, cation exchange capacity, and pH buffering. Organic matter is also a source of carbon (C) and energy for microorganisms.

Soil organic matter is comprised of decomposed plant and animal residues. It is a highly complex mixture of C compounds that also contain nitrogen (N), sulfur (S) and phosphorus (P). Organic matter is made up of humic substances and biochemical compounds. Humic substances are defined based on their solubility characteristics: humic acids are soluble in alkaline but not acidic solutions, fulvic acids are soluble in acid and alkaline solutions, and humic is the insoluble material that remains after humic and fulvic acid extraction [53]. Biochemical compounds include identifiable organic compounds such as organics acids, polysaccharides,

sugars and lipids. General properties of soil organic matter and their effect on soil are listed in **Table 2.7**

Table 2.7: Soil Organic Matter Properties and Their Associated Effects on Soil ^[53]

Property	Remarks	Effects on soil
Color	The typical dark color of many soils is caused by organic matter	May facilitate warming
Water retention	Organic matter can hold up to 20 times its weight in water	Helps prevent drying and shrinking; improves moisture-retaining properties of sandy soils
Combination with clay minerals	Cements soil particles into structural units called aggregates	Permits exchange of gases; stabilizes structure; increase permeability
Chelation	Forms stable complexes with Cu ²⁺ , Mn ²⁺ , Zn ²⁺ , and other polyvalent cations	Enhances availability of micronutrients to higher plants
Solubility in water	Insolubility of organic matter is due to its association with clay; also, salts of divalent and trivalent cations with organic matter are insoluble	Little organic matter is lost by leaching
Buffer action	Exhibits buffering in slightly acid, neutral, and alkaline ranges	Helps to maintain a uniform soil pH
Cation exchange	Total acidities of isolated organic matter fractions ranges from 300 to 1400 cmol/kg	Increase cation exchange capacity (CEC) of the soil; from 20 to 70% of the CEC of many soils (e.g., Mollisols) is due to organic matter
Mineralization	Decomposition of organic matter yields CO ₂ , NH ₄ ⁺ , NO ₃ ⁻ , PO ₄ ³⁻ , and SO ₄ ²⁻	Source of nutrients for plant growth
Combination with xenobiotics	Affects bioactivity, persistence and biodegradability of pesticides	Modifies application rates of pesticides for effective control

Soil organic matter can absorb trace elements pollutants such as lead (Pb), cadmium (Cd), copper (Cu), which will reduce the chance of contamination of surface waters and groundwaters. Another advantage is the adsorption of pesticides and other organic chemicals. This reduces the possibility of pesticide carryover effects, prevent contamination of environment, and enhance both biological and nonbiological degradation of certain pesticides and organic chemicals [53].

b. pH

Results of chemical tests are soil quality indicators which provide information on the capacity of soil to supply mineral nutrients, which is dependent on the soil pH. Soil pH is an estimate of the activity of hydrogen ions (H^+) and refers to a soil's acidity and alkalinity in the soil solution. It is also an indicator of plant available nutrients [55].

A high amount of H^+ corresponds to a low pH value and vice versa. The pH scale ranges from approximately 0 to 14 with 7 being neutral, below 7 (acidic), and above 7 alkaline (basic). Soil pH can affect cation exchange capacities (CEC) and anion exchange capacity (AEC) by altering the surface charge of colloids. A higher concentration of H^+ (lower pH) will neutralize the negative charge on colloids, thereby decreasing CEC and increasing AEC. The opposite occurs when pH increases [55]. High activity is not desirable and the soil may require liming with base cations Ca or Mg in order to bring the solution back to neutral.

2.7 Source of Soil Pollution

Municipal, industrial, or mines wastes, or dug-up contaminated soils or sediments may contain a wide range of pollutants, including metals, cyanide (CN^-), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), asbestos, methane (CH_4), ammonia (NH_3), and hydrogen sulphide (H_2S), which may pose threat to the surrounding, unpolluted soil [57].

Nowadays, releases of industrial pollutants into soil may also occur accidentally, for example as a result of road accidents or accidental spills from industrial installations. Liquid substances in particular can easily pollute large volumes of soil, because they easily penetrate and disperse in soil. [57].

2.7.1 Soil-oil Contamination

Petroleum hydrocarbons are one of the common examples of toxic organic chemicals, which enter the environment and in large volumes through numerous routes. The seepage from natural deposits is one of the major routes by which petroleum oil enters marine environments [58]. In recent years, leakage of gasoline from underground storage tanks primarily at automobile service stations and from pipelines has been experienced at an alarming rate. When these substances spill into the soil, they penetrate into the deeper level of soil where because of oxygen shortage anaerobic processes prevail

[59] [60]. After that, they adsorbed by to the soil particles until they reach groundwater, where they will dissolve in water, float on the water surface or sink to the bottom of aquifer. Any petroleum hydrocarbons that dissolve in water will then travel with the ground water to some extent. Light-end petroleum products, such as gasoline, are more volatile, and will tend to float on water, whereas the heavy-end petroleum hydrocarbons, such as heavy heating oil, will tend to sink [45].

The Persian Gulf War brought about some of the worst environmental pollution ever recorded as the result of oil spills. From August 1990 to February 1991, 240 million gallons of oil were spilled into the Arabian and Persian Gulf and in the Kuwait Desert Making this is the largest oil spill in history [61].

Seven hundred kilometers of Kuwait and Saudi Arabian coastline were polluted as well as an area of approximately 49 square kilometers in Kuwait Desert [62]. These oil spills were the result of deliberate damage done by Iraqi forces during their invasion of Kuwait. Crude oil was deliberately dumped into the gulfs and oil production, storage, and refining facilities formed huge oil lakes as deep 2 meters. Many of the oil wells were set on fire and this result in the aerial deposition of partially combusted oil particles and associated non-combusted products of the oil fires, which also polluted large areas. Along with this the oil quality was also degraded due to long exposure to harsh atmospheric condition which caused it to lose volatile hydrocarbons and experience other physical and chemical changes making its sale less profitable [63].

A variety of techniques have been successfully used to clean up the soil and groundwater contaminated with petroleum hydrocarbon, including pump and treat of groundwater, excavation of shallow contaminated soils, and vapor extraction. However, many of these technologies are costly and do not result in complete destruction of the contaminants [45]. Biological treatment, on the other hand, has been developed as one of the most promising treatment technologies for petroleum hydrocarbons. Therefore, there are three bioremediation techniques were used to restore the contaminated soil such as land farming, windrow composting piles and bioventing.

2.8 Microorganisms

Many microorganisms are also known as to degrade a wide range of aromatic compounds [64]. The degradation of polyaromatic hydrocarbons (PAH) by microorganisms depend to a large extent on their molecular weights among many others factor [65]. Microorganism is the term often used to describe a free living cell. That definition would include all prokaryotes, as well as the unicellular eukaryotes: protozoa, algae and fungi. Microorganisms are ubiquitous in the environment and are responsible for most of the cycling carbon, nitrogen, sulfur, phosphorus, and other minerals. Microscopic multicellular organisms and unicellular organisms (microorganisms) play particles

roles in bioremediation processes. Bacteria are nearly always the primary degraders, although in some cases fungi are important [66].

Bacteria play the largest role in biodegrading organic contaminants in soil and groundwater. Fungi, like bacteria, metabolize organic compound but do not compete well in most engineered remediation systems. In well-aerated soils both bacteria and fungi will be important, but in poorly aerated soils the bacteria alone are responsible for the biological and chemical changes taking places [67]. Protozoa scavenge particulate materials, including other microorganisms, a role similar to that of animals on a larger scale. Algae are photosynthetic and, like plants, can be used to provide oxygen to microbial systems to remove inorganic nutrients. However, the light sources required for the growth of algae are rarely feasible in bioremediation systems. A few biological treatment processes that take advantages of the potential contributions of algae and fungal treatment process under development for use with some hazardous material [66].

2.8.1 Bacteria as oil-biodegradable agent

Current evidence suggests that in aquatic and terrestrial environments microorganisms are the chief agents for the biodegradation of molecules of environmental concern, including petroleum hydrocarbons [68]. Bacteria and yeast appear to be the dominant degraders in aquatic ecosystems while fungi and bacteria are the main degraders in soil environments [69].

The degradation of both crude and refined oils seems to involve a consortium of microorganisms, including both eukaryotic and prokaryotic forms. The most common genera known to be responsible for oil degradation comprise *Nocardia*, *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Micrococcus*, *Arthrobacter*, *Corynebacterium*, *Achromobacter*, *Rhodococcus*, *Alcaligenes*, *Mycobacterium*, *Bacillus*, *Aspergillus*, *Mucor*, *Fusarium*, *Penicillium*, *Rhodotorula*, *Candida*, and *Sporobolomyces* [70].

Of the various petroleum fractions, n alkanes of intermediate length (C₁₀-C₂₀) are the preferred substrates and tend to the most readily degradable whereas shorter chain compounds are rather more toxic [71] [72] Longer chain alkanes known as waxes (C₂₀-C₄₀) are hydrophobic solids and difficult to degrade due to their poor water solubility and bioavailability [73]; branches chain alkanes are also degraded more slowly than the corresponding normal alkanes [71].

The degradation of PAH by microorganisms depends to a large extent on their molecular weights among many other factors [74]. Most of the literature concerning the microbial transformation of PAHs has centered on the lower-molecular weight compounds such as naphthalene, anthracene and phenanthrene, but more recent studies have describes the capabilities of other microorganisms to metabolize higher molecular weight compound, including the white rot fungus *Phanerochaete chrysosporium* [75]

Recently, a soil *Mycrobacterium* strain was also shown to be able to metabolize pyrene as the sole source of carbon and energy and its rate of metabolism was doubled by the addition of a solvent such as paraffin [76].

3. Conclusion

An ever increasing demand of fuels has been a challenge for today's scientific workers. The fossil fuel resources are dwindling day by day. The renewable fuel usage has also increased because of environmental concerns about the fuel oxygenate MTBE (Methyl tert-butyl ether) that has been eliminated in the U.S. from 2005 onwards. Biodiesel seems to be a solution for future. Demand for renewable fuels is increasing worldwide because of new legislation seeks to reduce our reliance on fossil resources. For example, EU directive 2009/28/EC, known as the Renewable Energy Directive, targeted for renewable in the transport fuel mix, of 10% by 2020, subject to the sustainability of production [77]. The ability of environmental microorganisms to degrade and metabolize fuel components is an intrinsic defense against pollution where fuels are spilled or leaked. Therefore, engineers and scientist have to initiate the current and future multi disciplinary challenge in order to ensure the sustainability of both environment and the society.

Acknowledgment

The authors gratefully acknowledge Multi-disciplinary Research Grant (MDR) UTHM for the financial support.

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