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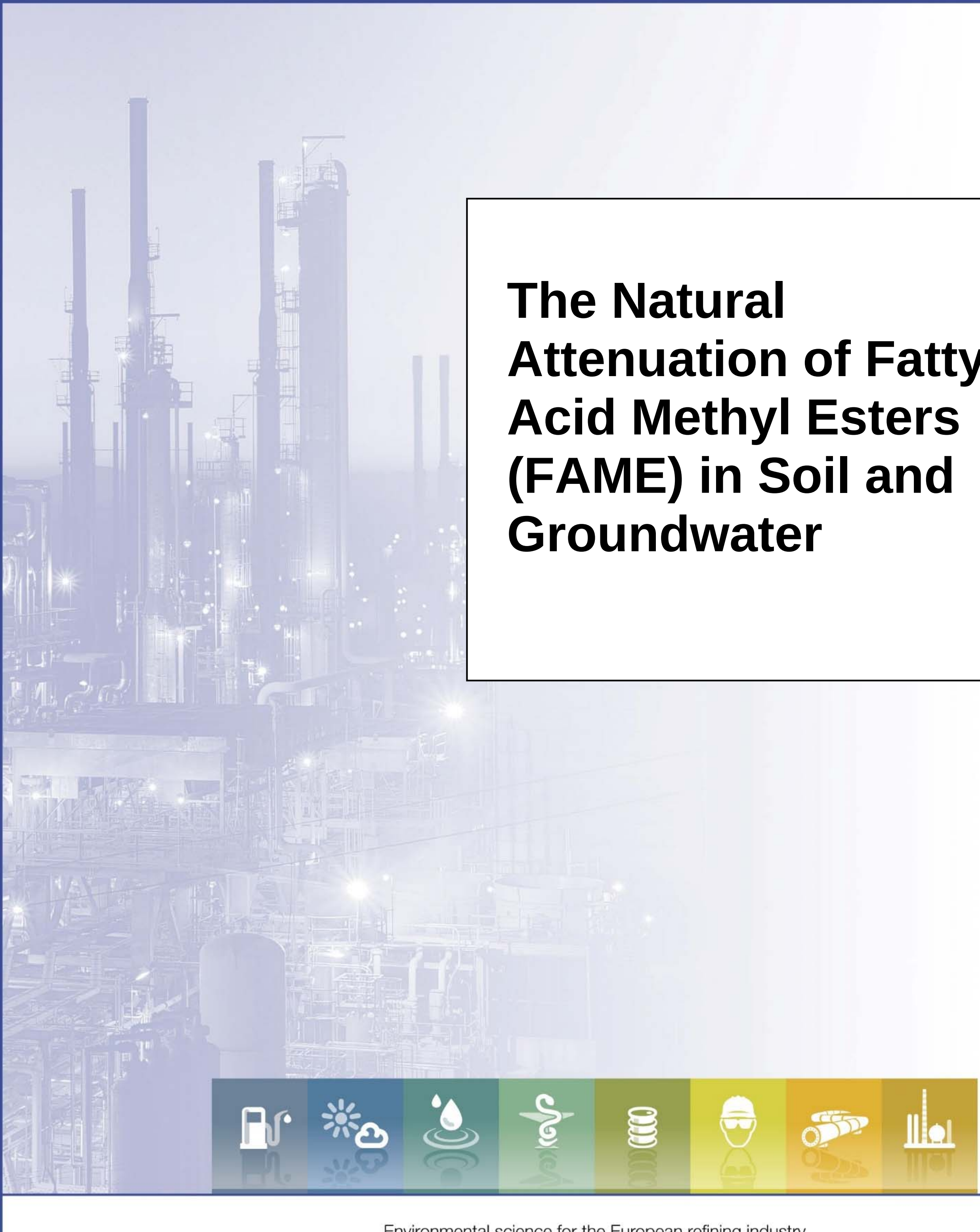

The Natural Attenuation of Fatty Acid Methyl Esters (FAME) in Soil and Groundwater

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The Natural Attenuation of Fatty Acid Methyl Esters (FAME) in Soil and Groundwater

Prepared for the CONCAWE Fuels Quality and Emissions Management Group by its Special Task Force, WQ/STF-33.

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ABSTRACT

With the increasing use of fatty acid methyl esters (FAME) in automotive fuels there is growing interest in the fate and effects of biodiesel, and biodiesel/ petroleum distillate blends in the environment. While biodegradation of FAME has been documented, uncertainty remains regarding how FAME partitions and degrades in the subsurface either alone, or in conjunction with other fuel components (e.g. hydrocarbons). A review of technical literature has therefore been completed to bring together the available data in this area.

The low solubility of FAME in groundwater means it is likely to behave as a light non-aqueous phase liquid (LNAPL) source with a relatively small region of influence. In this context, B5 or B20 biodiesel/petroleum blends may be expected to behave similarly to petroleum diesel in the subsurface. FAME does not appear to enhance the solubility of hydrocarbons as a whole, or individual components such as poly or mono-aromatic hydrocarbons. Individual FAME compounds have low aqueous solubility, low volatility and low mobility but the mechanisms of autoxidation and hydrolysis may result in the generation of more mobile but equally biodegradable components.

FAME is widely reported to be readily biodegradable under both aerobic and anaerobic conditions, although rates may vary from site to site as a function of ground conditions, electron acceptor concentrations and biodiesel composition. FAME appears to enhance the biodegradability of petroleum diesel at concentrations of B20 and higher, but this effect has not been demonstrated at field scale in the context of a subsurface release. At sites with limited electron acceptors and macronutrients (nitrogen and phosphate), microorganisms that degrade FAME have the potential to deplete available electron acceptors and nutrients resulting in an extended time for diesel biodegradation. As with other labile biofuels such as ethanol, anaerobic biodegradation of FAME has the potential to produce significant quantities of methane, which should be taken into account during the risk assessment of biodiesel and biodiesel/ petroleum diesel blend release sites

Overall, natural attenuation would appear to be significant in controlling the fate, behaviour and potential risks posed by biodiesel and biodiesel/ petroleum diesel blends. Significant attenuation mechanisms include sorption, auto-oxidation and biodegradation via a variety of redox processes: the exact role and contribution of each will depend on the nature of the release, the characteristics of the biodiesel and the environmental setting.

DEFINITIONS

For the purposes of this review the term “biodiesel” refers to 100% fatty acid alkyl ester (FAAE). However, where the type of FAAE is known this is stated to avoid ambiguity (e.g. FAME, soybean methyl ester). Pure petroleum diesel is referred to as diesel and where biodiesel/diesel blends are discussed the percentage of biodiesel is indicated (Bxx%), together with the type of FAAE present, if known.

KEYWORDS

FAME, Biodiesel, Natural Attenuation, Soil, Groundwater, Fatty Acid Methyl Esters

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SUMMARY

This report is a synthesis of the peer-reviewed scientific literature on the natural attenuation of biodiesel and biodiesel/ petroleum distillate mixtures in the subsurface. Much of the published literature concerns fatty acid methyl ester (FAME) due to its importance as a fuel blending component, with more limited data available on other fatty acid alkyl esters (FAAE), such as fatty acid ethyl ester. The report includes relevant data on biodiesel composition, the physical/ chemical properties of FAAE and also published data from laboratory and field-based studies of natural attenuation. The key conclusions from this review are as follows:

- The majority of fuels containing FAAE comprise a blend 5-20% FAME (B5 to B20) in petroleum diesel, with B100 (100% FAME) present only at fuel production and storage sites. The composition and properties of FAME within a given biodiesel vary according to the original FAME feedstock, the presence of additives and the blend of feedstock used in a commercial product.
- Individual FAME compounds are of low aqueous solubility, low volatility and low mobility. FAME, based on its low bulk density and aqueous solubility, is expected to exist as a light non- aqueous phase liquid (LNAPL) in the subsurface. A FAME LNAPL source would be relatively immobile, potentially long- lived (dependent on the volume of the release), but have a relatively small region of influence. In this context, a B5 or B20 FAME/petroleum diesel blend may be expected to behave similarly to petroleum diesel in the subsurface.
- The mixtures of FAME that have been studied in peer review literature do not appear to enhance the solubility of hydrocarbons as a whole or individual components such as poly or mono-aromatic hydrocarbons.
- Numerous laboratory studies have been conducted to investigate the biodegradation of FAME from various feedstocks under aerobic and methanogenic conditions. Under aerobic conditions mineralisation to carbon dioxide is typically observed and under methanogenic conditions methane is produced. A limited number of laboratory studies have provided evidence that biodegradation of FAME occurs through nitrate and sulphate reduction, with nitrate and sulphate depletion in response to FAME biodegradation observed at one field site. Although no laboratory studies were identified that evaluated biodegradation of FAME through iron or manganese reduction, increases in dissolved iron in response to FAME have been observed at the two field sites. These observations are consistent with FAME biodegradation through all major redox processes involved in natural attenuation.
- FAME is reported to increase aerobic biodegradation rates of n-alkanes in diesel at concentrations of B20 and higher but this effect, which is attributed to increased micelle formation, has not been demonstrated at field scale in the context of a subsurface release. At sites with limited electron acceptors and macronutrients (nitrogen and phosphate), microorganisms that degrade FAME have the potential to deplete available electron acceptors and nutrients resulting in an extended time for diesel degradation.
- As with other labile biofuels such as ethanol, anaerobic biodegradation of FAAE has the potential to produce significant quantities of methane. The degradation of FAME at the two field sites discussed in Section 7 led to the detection of high concentrations of methane in groundwater (30mgl^{-1}) and soil gas (up to 67%). These observations suggest that methane off-gassing should be taken into

account during the risk assessment of biodiesel and biodiesel/ petroleum diesel blend release sites.

- While studies support the overall conclusion that FAME is readily biodegradable under both aerobic and anaerobic conditions, the specific details – rate, observation of a lag period, extent of degradation, preferential degradation of specific FAME – varied from study to study. Site–specific assessment of natural attenuation processes, in accordance with lines-of-evidence based good practice on MNA, remains necessary to demonstrate MNA on a site by site basis.
- FAME has been reported to undergo relatively rapid auto-oxidation and hydrolysis in aqueous solution, with 5-10% conversion to free fatty acids and methanol over a 24 hour period. These more soluble, but equally biodegradable substances could increase the concentration of dissolved organic carbon in groundwater beyond that expected for the parent FAME. Confirmation of complete FAME biodegradation requires more than disappearance by GC because intermediates produced through auto-oxidation and hydrolysis are not detectable by standard GC methods for FAME and diesel. With the exception of methanol, these intermediates are not known to be toxic, but could continue to impact water quality. Additional work is needed to explore these effects.

Overall, natural attenuation would appear to be significant in controlling the fate, behaviour and potential risks posed by biodiesel. Significant attenuation mechanisms are likely to include sorption, autoxidation and biodegradation via a variety of redox processes: the exact role and contribution of each will depend on the nature of the release, the characteristics of the biodiesel and the environmental setting. Such attenuation may have secondary impacts on the degradation of other components of the biodiesel and may in itself generate undesirable effects such as excessive methane.

The literature contains relatively few studies of the fate and behaviour of biodiesel at field scale and few published case studies. In particular, there is limited data on the impact of FAME on the fate and transport of petroleum diesel and the production of methane. Additional examples of either controlled or accidental releases of biodiesel or biodiesel/ petroleum diesel blends, would enhance understanding of the biodegradation processes discussed above and the behaviour of these processes in different geologies.

1. INTRODUCTION

Fatty acid methyl esters (FAME) are a group of organic compounds that can be synthesised through the process of esterification of fatty acids with methanol (Energy Institute 2008 and Ginn et al 2009). FAME are of considerable environmental and economic importance as they are a key constituent of commercial “biodiesel” fuel, which may comprise neat FAME but more typically is a FAME/ petroleum diesel blend (Ginn et al 2009). With the increasing use of FAME in fuel, the likelihood of releases of FAME and biodiesel to the environment will increase over time. Effective management of such releases will require an understanding of the fate and transport of FAME and the impact of FAME on the fate and transport of diesel.

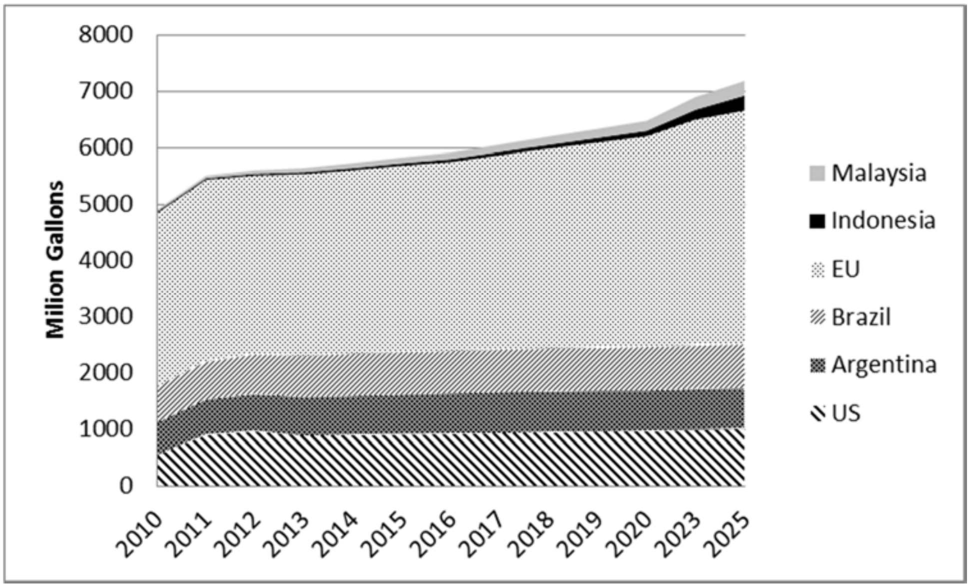
FAME may be sourced from a variety of feedstocks, including vegetable oils (rapeseed, soy, palm, sunflower and maize), animal fats (tallow, lard, poultry and fish oils) and waste oils and fats (used cooking oils) (CONCAWE 2009 and Ginn et al 2009). A number of methods are in use for the production of FAME and have been described in detail elsewhere (Ginn et al 2009; ITRC 2011; CONCAWE 2009 and Moser 2009). In the most common process, FAME are produced via the transesterification of the feedstock material through reaction with methanol in the presence of a catalyst. The resulting mixture contains FAME and glycerine (glycerol) and the latter is separated from the FAME prior to use.

Fuels containing FAME are recognised by the percentage of FAME in the mixture using the letter B followed by the percentage in the fuel. B100 is pure FAME and, while B100 can be used directly as a fuel, it is usually blended with petroleum-derived diesel to produce an amended biofuel (Prince et al 2008). Typically blends are 5, 7 and 20 % (V/V) biodiesel and are designated B5, B7 and B20. Biodiesel is internationally recognised as an alternative to conventional fuel, and a number of standards have been developed that specify the key properties of biodiesel (e.g. ASTM D6751, BS EN 14214). Globally, current mandates for blends of FAME with conventional diesel vary from B2 to B10 with the State of Minnesota planning a move to B20 (Smith 2014 & Lane 2013). In Europe, the maximum FAME content in EN590 diesel fuel is 7% (V/V) (B7), and a new standard is being developed (FprEN 16734) that allows blending of up to 10 % (V/V) FAME (B10) in accordance with the EN14214 specification.

Climate change and fuel security issues have resulted in an increasing interest in the manufacture and sale of renewable fuels globally (Fuller et al 2013). For biodiesel, this trend is driven primarily by legislation (e.g. The European Renewable Energy Directive (RED, 2009/28/EC) mandates the use of 10% renewable energy in road transportation and non-road mobile machinery by 2020) to reduce greenhouse gas emissions and enhance energy security.

Industrial scale production of biodiesel fuel has been undertaken in Europe since 1991 (EBTP 2011). Biodiesel production is predicted to increase globally in the coming decades as illustrated in **Figure 1.**

Figure 1: World Biodiesel Production Projections (FAPRI 2011))



Guidance on the safe use and handling of biodiesel has been developed both in Europe and the USA (CONCAWE 2009 & NREL 2009). However, there is also interest in understanding the fate and behaviour of biodiesel in the event of a release to the subsurface as either a result of accidents, leakages or spills (California EPA 2015). Considerable work has been undertaken to examine the fate of other biofuels such as ethanol (Morgan et al 2014) but far less research appears to have been published on biodiesel. Possible reasons for this include the higher prevalence of ethanol-based biofuels (FAPRI 2011), the low usage of biodiesel in the USA, and the understanding that FAME is non-toxic and readily biodegradable (US EPA 2004 & NBB 2012). While aerobic and anaerobic biodegradation of biodiesel has been documented, uncertainty remains regarding how biodiesel partitions and degrades in the subsurface and its effect on the fate of petroleum hydrocarbons. A review of technical literature has therefore been completed to bring together the available data in this area.

2. OVERVIEW OF NATURAL ATTENUATION

Natural attenuation has been defined as the "reduction in mass or concentration of a compound in groundwater over time or distance from the source of constituents of concern due to naturally occurring physical, chemical, and biological processes, such as; biodegradation, dispersion, dilution, adsorption, and volatilisation" (ASTM 1998).

The significance of natural attenuation processes in limiting the environmental impact and risk posed by subsurface releases of chemicals has been recognised in the last 20 years (Weidemeier et al 1995) and natural attenuation has been shown to be effective for a variety of contaminants including petroleum hydrocarbons and chlorinated solvents. Guidance and procedures for the quantification of natural attenuation have been published in many countries and monitored natural attenuation, as a remedial technique, is an integral part of risk based sustainable land management.

The focus of natural attenuation has typically been on the fate of dissolved phase or volatile contaminants as they migrate in the plume. More recently, the quantification of natural attenuation processes in the source zone in a process known as natural source zone depletion (NSZD) has gained increased interest (ITRC 2009 & Johnson et al 2013).

Processes of significance in natural attenuation include volatilisation, dilution, dispersion, adsorption and degradation. Abiotic and biotic degradation processes are considered the most significant and important as these processes result in contaminant mass reduction as well as reduction in concentrations.

Natural attenuation is typically evaluated using a lines-of-evidence approach (ASTM 1998 & Environment Agency 2000) that is based on the analysis and documentation of independent but converging lines of evidence including:

- Primary lines of evidence - Historical groundwater and/or soil chemistry data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points;
- Secondary Lines of evidence - Hydrogeological and geochemical data that can be used to demonstrate indirectly the type(s) of natural attenuation processes active at the site and the rate at which such processes will reduce contaminant concentrations to required levels; and
- Tertiary Lines of evidence - Data from field or microcosm studies (conducted in or with contaminated site media) that directly demonstrate the occurrence of a particular natural attenuation process at the site and its ability to degrade the contaminants of concern (typically used to demonstrate biological degradation processes only).

Monitored natural attenuation (MNA) is considered a potentially suitable remedial technique for biodiesel (Energy Institute 2008 & ITRC 2011).

The following sections review the available literature on FAME composition and properties that will influence the natural attenuation of FAME in the environment. The review also covers the effect of FAME on the natural attenuation properties of hydrocarbons present in biodiesel.

3. BIODIESEL COMPOSITION

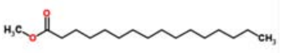
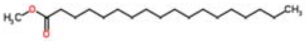
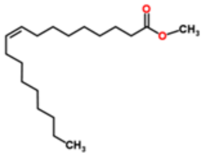
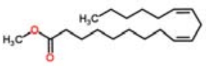
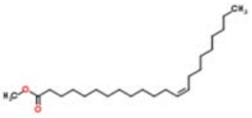
3.1. USE OF FATTY ACID ALKYL ESTERS IN BIODIESEL

The predominant fatty acid alkyl ester (FAAE) used in European biodiesel production is FAME, although in future other FAAE may also be used, such as fatty acid ethyl ester (FAEE). Work is underway to extend the test methods and specifications in EN 14214 to include FAEE, and to investigate the performance of FAEE-containing blends in engines and vehicles. The successful completion of this work is a prerequisite for accepting FAEE as a diesel fuel blending component. A further barrier to FAEE use in biodiesel is the higher price of ethanol compared to methanol. If methanol prices were to increase relative to ethanol, and if GHG emission reduction become a critical factor for legal compliance, then FAEE production could become an economically attractive alternative to FAME (Concawe, 2009).

3.2. FAME COMPOSITION IN BIODIESEL

FAME have the general molecular structure $CH_3(CH_2)_nCOOCH_3$ (saturated) and $CH_3(CH_2)_n(CH)_xCOOCH_3$ (unsaturated). Examples of FAME produced from the main vegetable oils used in biodiesel production are summarised in **Table 1**.

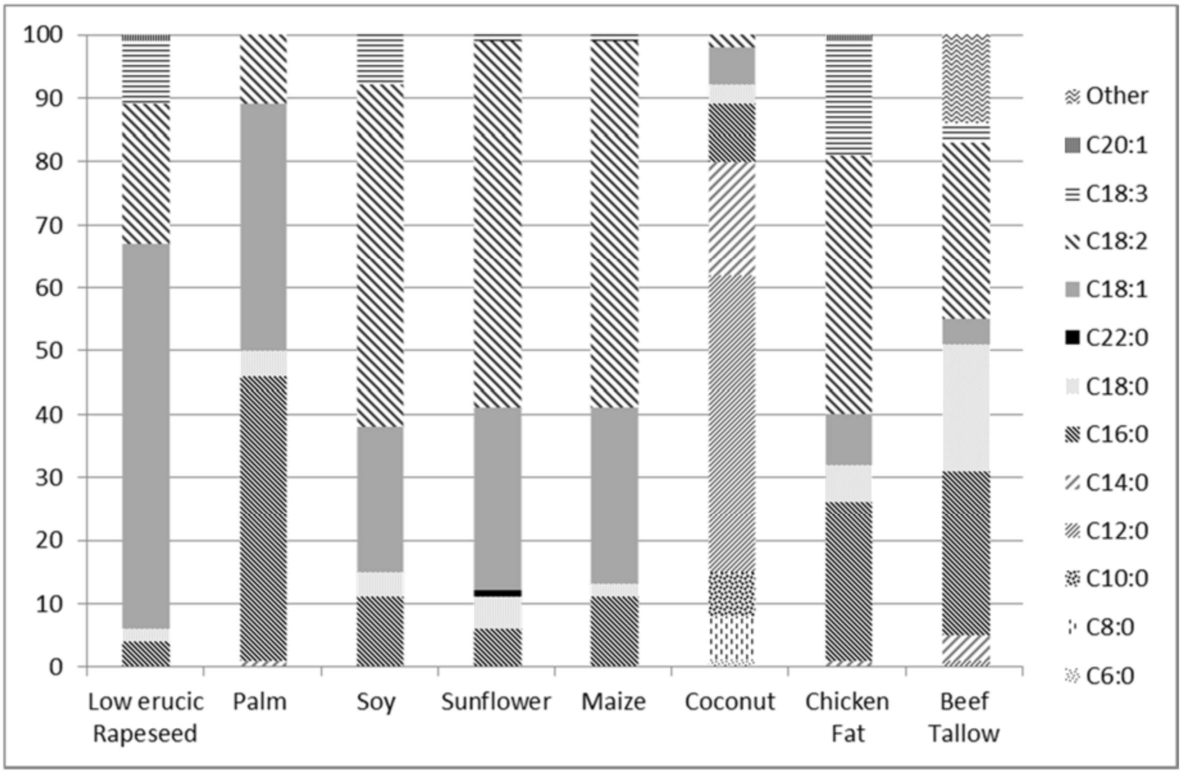
Table 1: Examples of FAME

Palmitic acid methyl ester (C16:0)	
Stearic acid methyl ester (C18:0)	
Oleic acid methyl ester (C18:1)	
Linoleic acid methyl ester (C18:2)	
Linolenic acid methyl ester (C18:3)	
Erucic acid methyl ester (C22:1)	

Note: Numbers describing each acid indicate the number of carbon atoms in the chain followed by the number of unsaturated carbon-carbon bonds in the chain.
2D images sourced from www.Chemspider.com

The composition of FAME will depend on a number of factors including the origin of the feedstock used and the manufacturing process (Energy Institute 2008). In addition, the genotype, growing seasons and growing conditions have all been found to affect oil content and fatty acid profiles (Hollebone et al 2009). **Figure 2** summarises the relative proportions of fatty acids present in various common feedstocks.

Figure 2: Typical fatty acid composition of FAME feedstocks (adapted from Moser 2009)



Generally, the source of feedstock oil is governed by geography and local market conditions, with, for example, rapeseed (low erucic) and sunflower common in Europe, soybean in the USA and canola (low erucic rapeseed) in Canada (Hollebone et al 2009 & ITRC 2011). In the USA, the three primary feedstocks are soybean (accounting for almost 70% of virgin plant based feedstock production in 2014), canola and yellow grease (US EIA 2015). In Europe, Concawe (2009) noted that FAME products sold for fuel blending are most commonly a mixture of different FAME products manufactured from different feedstocks, where the final FAME composition is a complex function of cost, availability and performance. In a recent study of biodiesel composition at 60 filling stations across Germany the source of the FAME in the biodiesel component included: rapeseed (53%), palm (25%), soybean (11%) and coconut (11%) (UFOP 2013).

3.3. BIODIESEL ADDITIVES AND IMPURITIES

The FAME blending component of biodiesel may contain small quantities of other substances, including contaminants and additives. Contaminants can include methanol, water, catalyst, glycerol, free fatty acids, soaps, metals and mono-, di- and triglycerides (Moser 2009). Permitted limits for impurities in B100 biofuel under standards EN-14214 and ASTM-D6751 are summarised in **Table 2**.

Table 2: Standard specifications for B100 fuel (100% FAME)

Property	**Units**	**EN-14214**	**ASTM-D6751**
Density at 15°C	g/cm^3	0.86 – 0.90	-
Viscosity at 40°C	mm^2/s	3.50 – 5.00	1.9 - 6.0
Flash Point	°C	101 min	93 min [1]
Cetane number	-	51 min	47 min
Sulphur	mg/kg	10.0 max	15.0 max [2]
Phosphorous content	mg/kg	4.0 max	10.0 max
Water content	mg/kg	500 max	500 max [3]
Acid number	mg KOH/g	0.50 max	0.50 max
Free glycerine / glycerol	% mass	0.02 max	0.02 max
Total glycerine / glycerol	% mass	0.25 max	0.24 max
Sulphated ash content	% mass	0.020 max	0.020 max
Methanol content	% mass	0.20 max	0.20 max [4]
Monoglycerides	% mass	0.70 max	0.40 max
Diglycerides	% mass	0.20 max	-
Triglycerides	% mass	0.20 max	-
FAME content	% mass	96.5 min	-
Linolenic acid methyl ester	% mass	12.0 max	-
Carbon residue	% mass	-	0.050 max
Iodine value	-	120 max	-
Oxidation stability at 110°C	Hours	8.0 min	3.0 min
Copper corrosion	Degree of corrosion	Class 1 [5]	No. 3 [6]
Distillation 90% recovered	°C	-	360 max

[1] closed cup (ASTM D93)
[2] S15 grade
[3] water + sediment (ASTM D2709)
[4] or min. flash point 130°C (ASTM D93)
[5] EN-ISO 2160
[6] ASTM D130

The use of additives to address fuel performance issues is ubiquitous in biodiesel and diesel formulation (Moser 2009). Biodiesel in particular requires a variety of additives to address oxidative instability, cold-flow limitations, microbial contamination, increased water affinity, and increased nitrous oxides (NOx) emissions (Riberio et al 2007). Additives can include antioxidants, biocides, cold flow enhancers, cetane enhancers, NOx reducers, water dispersants and anti-foaming agents (Hodam 2008). Given the varying nature of source oils and additives combinations, greater than 1,500,000 biodiesel formulations are possible (Hodam 2008).

Common antioxidants are a mixture of synthetic and natural products and include pyrogallol, propylgallate, tert-butyl hydroquinone (TBHQ), butylated hydroxy toluene (BHT), tocopherol and phenylenediamine. Hindered phenols are the most commonly used family of antioxidants and over 40 alternatives are currently marketed in Europe (AGQM 2014). The recommended concentration for antioxidant use varies from 200 – 3000 milligrams per litre (mg l^{-1}) and varies by anti-oxidant and FAME type (Dunn 2005).

According to Ginn et al 2009, biocides are too expensive to be widely employed in the upstream distribution process and a concern exists of creating microbial resistance. Biocides are, therefore, used on an “as needed” basis in the distribution chain where microbial contamination is a problem. The active ingredients in biocides are typically isothiazols, but methylene bis(thiocyanate) (MBT) and nitromorphalines are also used (Ginn et al 2009).

Additives evaluated as cold flow enhancers include ethylene-vinyl acetate copolymers, polymethacrylates and styrene–maleic anhydride copolymers (Riberio 2007).

4. PHYSICAL PROPERTIES

4.1. FAME PHYSICAL PROPERTIES

FAME have the appearance of a colourless to yellow/ brown liquid with little odour. The physical properties of FAME relative to their primary use as a fuel have been extensively studied (Knothe 2005) and the composition of FAME within an individual feedstock have been shown to influence strongly the properties of biofuels (Sanford et al 2009 & Knothe 2012).

In the context of environmental fate, the key physical properties of concern include density, viscosity, solubility, the Octanol-Water partition coefficient (K_{ow}), Henry's Law constant and vapour pressure. When using such data to predict environmental behaviour it is important to bear in mind that biodiesel is a complex and variable mixture of both FAME and other compounds, and that test results may be biased by trace components. For example the measured solubility may not reflect that of the major FAME blending components. This issue is discussed further in the text below.

An additional consideration is that biodegradation of FAME in the environment, as discussed in a later section, may result in the formation of other compounds, such as free fatty acids, that could modify its physical properties.

Alptekin & Canacki (2008) report on the measurement of the density and viscosity of six FAMEs produced from sunflower, canola, soybean, cottonseed, corn oils and waste palm oil. Density measurements varied from 0.87 to 0.88 g cm^{-3} as compared to typical European diesel densities (0.820 – 0.845 gcm^{-3} (EN 590)) and viscosity varied from 3.97 to 4.34 mm^2s^{-1} as compared to the diesel (2.00 – 4.50 mm^2s^{-1} (EN 590)). These properties of FAME are in, or close to, the range reported for conventional European EN 590 diesel fuel.

Ginn et al (2009) noted that no measurements of either the K_{ow} or vapour pressures for biodiesel fuels at ambient environmental temperatures have been reported though some measurements for individual components are available. A number of these properties are difficult to measure though some experimental data exists for some compounds such as n-octanol-water partition coefficient, aqueous solubility and Henry's law constant (Krop et al 1997) and volatility (Freitas et al 2012).

For the purposes of this review, mirroring the approach undertaken in Energy Institute review (2008), a simple assessment of the physio-chemical properties of individual FAME compounds has been undertaken using the US EPA Estimation Program Interface (EPI) Suite (US EPA 2014) and the results are presented in Table 3. Where available, the table also includes measured water solubility values reported by Krop et al 1997.

Table 3: Estimated and observed physiochemical properties of some individual FAME

Name	CAS No.	Molecular Weight	Estimated water solubility from Log K_{ow} mg l^{-1} 25°C	Measured water solubility mg l^{-1} 22°C	Henry's Law constant estimate atm-m^3mole	Log K_{ow} estimate	Vapour pressure estimate mmHg 25°C
Stearic acid methyl ester	112-61-8	298.5	$9.3 x10^{-4}$	$3.0x10^{-4}$	$1.4x10^{-2}$	8.4	$3.3x10^{-5}$
Erucic acid methyl ester	1120-34-9	352.6	$6.6x10^{-5}$	-	$7.8x10^{-3}$	9.3	$1.1x10^{-6}$
Linoleic acid methyl ester	112-63-0	294.5	$1.9x10^{-2}$	$2.1x10^{-2}$	$1.9x10^{-3}$	6.8	$9.9x10^{-5}$
Linolenic acid methyl ester	301-00-8	292.5	$5.8x10^{-2}$	$9.2x10^{-2}$	$2.1x10^{-4}$	6.3	$3.1x10^{-5}$
Oleic acid methyl ester	112-62-9	296.5	$5.5x10^{-3}$	$4.4x10^{-3}$	$8.0x10^{-3}$	7.5	$1.6x10^{-4}$
Palmitic acid methyl ester	112.39-0	270.5	$9.1x10^{-3}$	$4.0x10^{-3}$	$1.9x10^{-2}$	7.4	$4.8x10^{-4}$

Overall, and consistent with an earlier review (Energy Institute 2008), the aqueous solubilities of individual FAME compounds are low (<0.1 mgl-1) and FAME have very low volatility and a high affinity for organic matter with a minimum Log K_{ow} of 6.29. FAME are, therefore, likely to be strongly sorbed to natural organic matter and their transport significantly retarded in soil and aquifer systems.

In its review of biofuels, the ITRC summarised and compared the key properties of biodiesel in relation to conventional diesel, gasoline and ethanol. These are summarised in **Table 4**.

Table 4: Comparison of selected fuel component properties (adapted from ITRC 2011)

	Density (g/cm^3)	Water solubility (25°C mgl^{-1})	Henry's law constant (unitless)	Log Kow	Vapour Pressure (25°C mm Hg)
Diesel	0.820 - 0.845*	5**	$2.4x10^{-3}$ to $3.0x10^{+2}$	3.3 to 7.06	2.12 to 26.4
FAMEs	0.87 - 0.88***	$1.2x10^{-3}$ to $2.1x10^{-2}$	$6.4x10^{-3}$ to 1.3	6.29 to 8.35	$8.63x10^{-6}$ to $3.74x10^{-4}$
Benzene	0.88	1800	0.22	2.13	75
Butanol	0.81	$7.x10^{+4}$ to $8.7x10^{+4}$	$3.7x10^{-4}$ to $5.0x10^{-4}$	0.83 to 0.88	0.42 to 11.8
Ethanol	0.79	infinite	$2.1x10^{-4}$ to 2.6x10-4	-0.16 to -0.31	59

Footnotes:
* European standard EN590 for automotive diesel fuel
** The solubility of diesel reported in the above table is reported to be higher in some references for example Hollebone et al (2008) the "water-accommodating fraction" has a range of 16 to 45 mg/L +/5 mg/L
*** FAME density range reported by Alptekin & Canacki (2008)

The ITRC noted that of the fuel components considered FAME was by far the least soluble in water and the least mobile, either in the aqueous or vapour phase.

The values of aqueous solubility reported for FAME in **Table 4** are in contrast to other values reported in literature and may reflect the variety of methodologies that have been employed to undertake the measurements as well as the mixture of FAME present. For example Von Wedel (1999) reported that the saturation concentration of biodiesel in fresh water was 14 mgl^{-1} at a temperature of 17°C, though the biodiesel in question and analytical methodology used was not reported. Ginn et al 2009 noted that biodiesel is on average 15 to 25 times more soluble than diesel. Using data derived from an earlier study, Chiaranda

(2011) reported solubilities of individual FAME in the range 2.9 to 7.2 mgl^{-1} and effective solubilities (calculated using Raoult's Law) in a soy-based B100 biodiesel of between 0.036 and 0.5 mgl^{-1}. Toso (2010) reports the solubility of FAME in the range 10-20 mgl^{-1}. However, comparison of the reported solubilities with the derived values is difficult without understanding the biodiesel in question and the analytical methodologies used. FAME like fatty acids have a propensity to aggregate and solubility measurements may be influenced by sample preparation, additives, sampling methodology, analytical methodology and other factors.

As previously mentioned, FAME is a complex and variable mixture of organic compounds and may contain traces of compounds that have a higher solubility in water. During solubility testing these trace components may partition from the biodiesel into the aqueous phase, resulting in a test result that over-predicts the solubility of the major FAME components. For further discussion of published literature highlighting this effect, see the later section “FAME dissolution in water”.

4.1.1. Other physical properties of FAME of relevance to natural attenuation

An important physical characteristic of FAME that has relevance to their fate and behaviour in the subsurface is their susceptibility to oxidation when exposed to oxygen in ambient air (Knothe 2007). The presence of FAME makes biodiesel more susceptible to auto-oxidation than conventional diesel. Hancock and Leeves (1989) noted that unsaturated FAME have the almost unique ability to react with oxygen to generate hydroperoxides. Salam et al (2012) described the process as a free radical reaction divided into initiation, propagation, branching and termination steps that are initiated by free radicals frequently of unknown origin. Reactions of hydroperoxides include rearrangement to products of similar molecular weight, fission to give shorter-chain compounds (aldehydes, ketones and acids including acetic and propionic acids) and dimerization to give higher molecular weight materials (Knothe 2007). For example, Yassine et al (2012) identified methanol, hexanal, n-butyl acetate, diethylene glycol monobutyl ether, and diethylene glycol monobutyl ether acetate as auto-oxidation products of soybean FAME. Factors influencing auto-oxidation and the nature of reactions has been described in detail by Knothe (2007) & Salam et al (2012) and include factors such as elevated temperature, the presence of light or extraneous materials such as metals or initiators. Oxidation, since it specifically influences biodiesel quality, has been the subject of considerable research: European and certain US biodiesel standards (ASTM D6751 for B100) include an oxidative stability specification. Nevertheless autoxidation may have an important role in natural attenuation around plume fringes, but is an area of uncertainty.

Besides oxidation caused by exposure to air (oxygen), biodiesel is also potentially subject to hydrolytic degradation due to the presence of water (Knothe 2007). In absence of measured hydrolysis rates, DeMello et al 2007 assumed that a base catalysed pathway would control FAME hydrolysis and predicted base catalysed hydrolysis half-lives of 7 years at pH 7 and 70 years at pH 6 (25°C) and 3 years and 19 weeks at pH 7.4 and 8.3, respectively. The latter were considered upper bound estimates (DeMello et al 2007) and may be significant in the context of natural attenuation where ground water may typically be in the range pH 6-8.

4.2. PROPERTIES OF ADDITIVES

As indicated above, commercial biodiesel may be treated or supplemented with a variety of additives to improve storage and performance. The physical and chemical properties of these additives do not appear to have been systematically studied or reported in the context of potential influences on natural attenuation of FAME.

The Energy Institute (2008) reported the physical and chemical properties of a number of biodiesel antioxidants including Tocopherol, TBHQ, BHT and phenylenediamine. Tocopherol (naturally occurring vitamin E) and BHT were considered unlikely to have significant migration potential due to their low solubility in water and high affinity for organic matter. The migration potential for TBHQ and phenylenediamine was considered to be greater based on their higher solubility (748 mgl^{-1} and 40,000 mgl^{-1} respectively).

The active ingredients in the biocide Kathon FP 1.5 are a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one. Both substances are reportedly highly soluble in water and considered mobile but have reported aerobic and anaerobic half-lives of less than 1.4 day and less than 0.2 days, respectively (Dow, 2011).

The potential influences of additives on biodiesel properties and biological degradation is worthy of further research.

5. BIODEGRADATION OF FAME

Biodegradation is generally the major mass removal process involved in natural attenuation of organic compounds in the environment. Since the fatty acid components of FAME are natural products and ubiquitous components of cellular membranes, a wide array of naturally occurring microorganisms in soil and groundwater environments are capable of their transport and metabolism. Although methyl esters of fatty acids are not commonly found in nature, many fatty acids exist in organisms as glycerol esters (i.e. mono-, di- and triglycerides). For an understanding of the natural attenuation of FAME through biodegradation, more specific information on degradation processes is needed such as which terminal electron acceptor processes (TEAPs) will support microbial degradation of FAME, whether microorganisms capable of biodegradation of FAME are commonly found in soil and groundwater, and whether all FAME components are similarly amenable to biodegradation.

Numerous laboratory studies have been conducted to investigate the biodegradation of FAME from various feedstocks using different electron acceptors. When assessing these studies, it is important to note several aspects of the tests - the source of FAME, the microbial inoculum, whether inorganic nutrients have been added, and the analytical testing used to monitor disappearance/ degradation of FAME.

FAME - FAME utilised in the laboratory studies reported in the literature came from various feedstocks and were either obtained commercially or synthesised in the laboratory. Some investigators only reported the use of “biodiesel” without specifying the feedstock or provided only limited information on the source. Few researchers reported the presence or absence of additives such as BHT or TBHQ.

Microbial Inoculum - Several studies have used activated sludge or other inocula from a waste water treatment plant. These inocula are not typical of soil and groundwater environments, have high biomass, and have generally been acclimated to fatty acids in the form of lipids due to their presence in waste waters. Other studies have used soil, groundwater or surface water as the source of the inoculum. These environmental media would be expected to have comparatively low biomass and may or may not have been previously exposed to FAME or petroleum. In some cases, microbial cultures were isolated from one of these media and grown on petroleum, FAME or other substrate. The biomass of these inocula varied based on culture density and addition volume.

Nutrient Amendment – Test microcosms used either unamended soil, groundwater or surface water; water and/or soil amended with inorganic nutrients (nitrogen and phosphorus, or a standard mineral salts medium), or with a rich medium containing yeast extract or other carbon substrate in addition to mineral salts.

Chemical Analysis – The analytical technique used to monitor biodegradation has an influence of the results. Many studies used either the production of carbon dioxide or methane to monitor mineralisation of FAME or gas chromatography (GC) to monitor the disappearance of individual FAME compounds. Production of carbon dioxide or methane provides evidence of complete mineralisation, but does not provide information on the initial steps in degradation. Disappearance of FAME components as measured by GC has the potential to provide more detailed information. However, GC for FAME alone cannot be used to determine biodegradation since the abiotic transformations that FAME undergo, such as hydrolysis, auto-oxidation and polymerisation discussed earlier, will also result in disappearance of FAME from the GC results. A few studies have used other analytical techniques such as liquid chromatography for intermediates and infrared absorbance. No studies that used Compound-Specific Isotope Analysis (CSIA) were identified, although

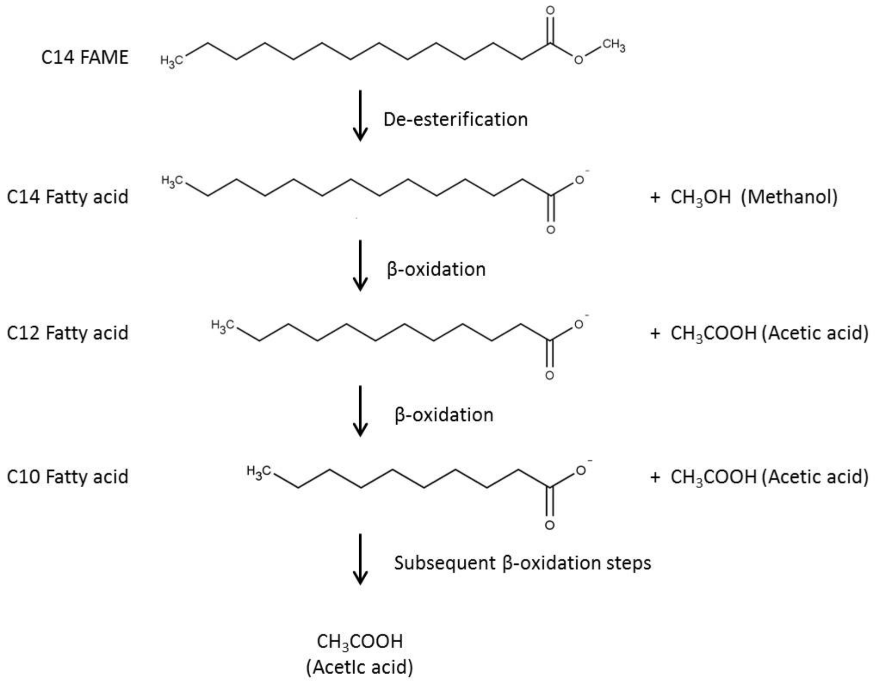
CSIA for individual FAME is offered commercially (Stable Isotope Facility at the University of California at Davis).

The following sections discuss studies on the general metabolic pathways for fatty acids and fatty acid methyl esters and studies looking at biodegradation under both aerobic and anaerobic TEAP processes.

5.1. GENERAL METABOLISM OF FATTY ACIDS AND THEIR METHYL ESTERS

FAME molecules are similar in structure to their parent fatty acid esters with the exception of the transesterification of the carboxylic group with methanol replacing glycerol to form a methyl ester. As discussed in a previous section, the ester function impacts physical properties and is also expected to have an effect on biodegradation. The studies that have looked at the catabolism of FAME have indicated the biodegradation pathway for FAME molecules is a multistep process that is similar under both aerobic and anaerobic conditions. FAME are first de-esterified to form free fatty acids and methanol (Souza et al 2007; Stolz et al 1995; Aktas et al 2010). The free fatty acids then undergo sequential removal of two-carbon components through a process known as "β-oxidation" as shown in **Figure 3** (Stolz et al 1995; Aktas et al 2010).

Figure 3: General Pathway for Metabolism of FAME



This pathway has not been well studied for FAME, but generally follows the well-known biodegradation pathway for naturally occurring fatty acids and fatty acid esters (i.e. glycerides), as modeled by standard pathway prediction tools such as the EAWAG-BBD Pathway Prediction System (http://eawag-bbd.ethz.ch/predict/). Metabolism of glyceride esters starts with de-esterification to form free fatty acids and glycerol by esterase enzymes known as lipases, which have been found in all studied microorganisms (White et al 1968). Lipases may also be involved in removal of the methyl ester group of FAME since the use of lipases as biocatalysts for the transesterification of oil feedstocks to produce FAME indicates that lipases can participate in esterification/de-esterification reactions involving fatty acid methyl esters (Ghaly et al 2010). Stolz et al 1995 found that bacterial strains that were capable of growth on FAME produced an esterase enzyme to cleave the methyl ester group (although the data were not shown). In addition, the de-esterification step for FAME may also occur through abiotic hydrolysis, as discussed earlier.

The β-oxidation pathway for fatty acid degradation was first discovered by Franz Knoop (1904) and is known to occur in both bacteria and mammalian cells. Souza et al (2009) reviewed the cyclic process of β-oxidation in the facultative anaerobic bacterium *Escherichia coli*. Through a series of enzymatic steps, the first two carbons are cleaved from the fatty acid with the production of acetic acid (in the form of acetyl-CoA) and a new two-carbon shorter fatty acid, which then continues the cycle by undergoing another round of β-oxidation. If the fatty acid is unsaturated (e.g. oleic acid (C18:1) or lineoleic acid (C18:2)), then additional enzymatic steps may be required to isomerise, or isomerise and reduce (hydrogenate), the double carbon bond prior to continuation of the β-oxidation cycle. Although *E. coli* carries out β-oxidation of fatty acids under both anaerobic and aerobic conditions, two different sets of enzymes are involved (Campbell et al 2003). While *E. coli* is a gut bacterium and not native to soil and groundwater ecosystems, intermediates consistent with degradation of FAME through β-oxidation have been detected in laboratory studies on aerobic and anaerobic bacteria isolated from environmental media, as discussed below. A few of these FAME-degrading bacteria from the environment have been identified (see **Tables 5** and **7**) but more work is needed to identify the full enzymatic pathways for fatty acid degradation by these bacteria.

In a study looking at the degradation of FAME derived from soybean oil by several environmental inocula under anaerobic conditions (sulphate reduction and methanogenesis), Aktas et al (2010) observed the formation of a series of lower carbon-chain length fatty acids as intermediates. The degradation of the soybean FAME, which is composed primarily of FAME molecules of even-number carbon chain lengths (including linoleic, oleic, stearic and palmitic methyl esters), produced a series even-number carbon chain fatty acids that ranged from C24 (tetracosanoic acid) to C6 (hexanoic acid), as would be expected from the β-oxidation pathway. Lesser amounts of odd-numbered carbon chain fatty acids (C23 to C13) were also detected and were assumed to be due to the β-oxidation of the odd-number carbon chain FAME, although no specific data were presented to support this conclusion. The data from the study also did not provide information on whether unsaturated fatty acids were hydrogenated prior to undergoing β-oxidation or were degraded faster or slower than saturated fatty acids of the same chain length.

Given the diversity of microorganisms present in soil and groundwater, the β-oxidation pathway may not be the only one involved in FAME biodegradation. Alkanes, which have long hydrophobic alkyl chains similar to FAME but are lacking the more polar ester group, also undergo β-oxidation after conversion to fatty acids (Rojo 2009; Rojo 2010). Under aerobic conditions, alkanes are generally first oxidised by the addition of a terminal hydroxyl group, which is subsequently oxidised to an aldehyde and then to a carboxylic acid group to form a fatty acid. Hydroxylation can also occur at a subterminal carbon position. Under anaerobic conditions, another pathway involving the addition of fumarate to form an alkyl succinate has been postulated (Rojo 2010). Although not documented in the literature, reactions such as hydroxylation or carbon addition under either anaerobic or aerobic conditions are also possible for FAME given the structural similarities of the alkyl chain with alkanes, and may produce intermediates other than simple fatty acids.

5.2. LABORATORY STUDIES OF FAME DEGRADATION

The biodegradation of FAME has been investigated through laboratory studies under both aerobic and anaerobic conditions. As discussed earlier, these studies have been conducted using various FAME, inocula, test conditions and analytical methodology.

5.2.1. Degradation under Aerobic Conditions

Laboratory testing has provided strong evidence for the biodegradation of FAME under aerobic conditions with mineralisation to carbon dioxide. Over 20 studies were identified that evaluated the aerobic biodegradation of FAME, as summarised in **Table 5**.

Table 5: Summary of Aerobic Laboratory Studies of FAME and FAME-Diesel Blends

Reference	Source of Inoculum	Media	Measurement	FAME and Other Substrates	Reported Results
Peterson & Reece, 1994	Not specified; however, BOD tests generally use sewage sludge and shake flask test generally use a weak bacterial inoculum	Aqueous solution (not further described) No abiotic control reported	BOD Measurement for shake flask test not described, but is generally DOC	Rapeseed methyl esters (RME) and rapeseed ethyl esters (REE) (likely synthesized at the University) Diesel Dextrose	High BOD for RME and REE In the shake flask test, 95% biodegradation of RME and REE within 28 days, as compared to 40% of diesel; rate of degradation of RME similar to REE, but faster than dextrose and diesel
Stoltz *et al.* 1995	Pond or lake water Top soil Culture grown on soybean FAME	Mineral salt medium	GC Methanol (by Draeger tubes)	Soybean FAME	100% FAME disappeared within 7 days with biomass and methanol production; nine aerobic FAME-degrading bacteria were isolated; detected esterase activity
Zhang *et al.* 1998	Organic-rich soil and aerated activated sewage sludge (unacclimated to FAME)	Yeast extract, amino acids, minerals	CO_2 production GC	Soybean and rapeseed FAME; soybean and rapeseed ethyl esters (SEE and REE) synthesized at the university using transesterification Neat rapeseed oil, neat soybean oil Phillips 2- reference diesel Blends of REE and diesel	In 28 days, 85.5% soybean FAME and 88.5% rapeseed FAME, were mineralized to carbon dioxide as compared to 87.8% for dextrose, 76.0 to 78.5% for the neat oils and 26.2% for diesel; as measured by GC, 100% of rapeseed ethyl ester disappeared within 3 days
Pasqualino *et al.* 2006	Two different activated sludge samples	Water with mineral media	CO_2 production	FAME from waste cooking oil (from BIONET Europa) Diesel and gasoline from fuel station Blends of waste cooking oil FAME with diesel and gasoline	In 28 days, almost 100% of the FAME was mineralized to carbon dioxide, while diesel and gasoline were mineralized 50% and 56%
Makareviciene & Janulis 2003	Unspecified bacterial culture	natural water	Infrared absorbance	Rapeseed methyl ester Rapeseed ethyl ester Diesel	In 21 days, about 98% of RME and REE had degraded, as compared to 61% of diesel
Schleicher *et al.* 2009	Culture isolated from uncontaminated soil	Water with nutrients	Acid number by titration Microbial enumeration	Rapeseed methyl ester (commercial) Diesel B5, B10, B20 blends of the above	Increase in free fatty acid content when rapeseed methyl ester was incubated with a microbial culture Higher microbial numbers in B100 incubated under anaerobic than aerobic conditions
Russell *et al.* 2005	Potting soil	Potting soil	GC	Soybean alkyl esters (ester not specified)	In 28 days, 80 to 90% of the soybean alkyl ester had disappeared
Peterson & Moller 2005	Sewage sludge	Aqueous	BOD by EPA Method 405.1	Soy bean and rapeseed methyl esters Rapeseed ethyl esters Neat soybean oil and rapeseed oil Phillips 2-D reference Diesel	BOD for methyl and ethyl esters and neat oils range from 1.5 to 1.7 x 10^6 mgl-1, as compared to 0.4 x 10^6 mgl-1 for petroleum diesel
DeMello *et al.* 2007	Seawater (unacclimated to FAME)	Seawater	GC	FAME (parent oil not specified, but containing C13 to C18) Commercial diesel B20 blend of the above	90% degradation of FAME within 21 days, compared to no loss in abiotic control

Mariano *et al.* 2008	River water, or soil from a petrol station with low fuel concentration	River water or soil from petrol station	Carbon dioxide (CO2)production	Laboratory prepared castor oil alkyl ester blended with diesel to B5 and B20. Commercial diesel and B2 (biodiesel component not specified)	CO_2 production from castor oil alkyl ester was 1.6-fold higher than for diesel
Owsianiak *et al.*2008	Microbial consortium isolated from a crude oil site; identified by 16SrRNA to contain *Pseudomonas alcaligenes, Ochrobactrum intermedium, Sphingobacterium sp., Pseudomonas putida, Klebsiella oxytoca,* *Chryseobacterium sp.* and *Stenotrophomonas maltophilia*; maintained in lab.	Mineral medium at pH 7	GC	Commercial rapeseed methyl esters Diesel (EN 590:2004) B5, B10, B20 etc. blends of the above prepared in the laboratory	Within 7 days, 76% disappearance of RME, as compared to 42% of diesel
Cyplik *et al.* 2010	Microbial consortium isolated from crude oil site under aerobic conditions containing *Achromobacter sp., Alcaligenes sp., Citrobacter sp., Comamonadaceae, Sphingobacterium sp., Pseudomonas* sp., and *Variovorax sp.*	Mineral medium at pH 7	GC	Commercial rapeseed methyl esters Diesel (EN 590:2004) B20 blends of the above prepared in the laboratory	Within 5 days, complete disappearance of RME, as compared to 10% disappearance of RME in the abiotic control and 20% diesel
Prince *et al.* 2008	Rainwater from detention pond (unacclimated to FAME)	Pond water plus mineral salt medium	GC	Commercial soybean methyl esters B20 (soybean FAME)	Half-lives for individual FAME (palmitate, stearate, oleate and linoleate methyl esters) ranged between 2.1 and 2.6 days
Ginn *et al.* 2012	Silty-loam soil	Soil slurry with mineral salts medium	GC with mass spectrometry detection CO2 production	Soybean methyl esters Animal fat methyl esters ultra-low sulphur diesel, B20 blends, with and without antioxidant (BioExtend containing TBHQ) and biocide additive	Carbon dioxide production was observed for a duration of 28 to 30 days, with no carbon dioxide production in the absence of the inoculum (soil) No significant effect of additives on aerobic biodegradation
Vauhkonen *et al.* 2011	Activated sludge, or Groundwater	Mineral media, or groundwater	Oxygen uptake (OECD 301F test)	Rapeseed methyl esters (commercial) Rapeseed oil	In 28 day, 60.8% RME and 65.8% rapeseed oil were biodegraded with a sewage sludge inoculum; only 19% RME and 9.9% rapeseed oil biodegraded with groundwater as the inoculum
Elazhari-Ali *et al.* 2012	Sandy soil	Wet sandy soil, with and without inorganic nutrients (N, P) Oxygen	GC of hydrocarbons	B20 made from commercial rapeseed FAME and 12 hydrocarbons blended in the laboratory to resemble gasoline or kerosene	Toluene was removed at essentially the same rate from the hydrocarbon-only mixture and B20 in the presence of added nutrients, but more rapidly from the hydrocarbon-only mixture (vs B20) in the absence of added inorganic nutrients.

Meneghetti *et al.* 2012	Two soils – clayey soil and sandy soil	Soil with and without fertilizer	CO_2 production GC	Vegetable oil methyl esters (source not specified)	Reductions of 16.8% in clayey soil and 58.5% in sandy soil were observed by GC in the absence of fertilizer after 110 days; higher percentage reductions were observed in the presence of fertilizer (59.8% in clayey soil and 90.4% in sandy soil Production of CO2 was reported, but appeared to be only a small proportion of the FAME
Yassine *et al.* 2013a	Culture isolated from aeration tank of wastewater treatment plant and gasoline and triglyceride degrading cultures; acclimated to diesel and FAME	Aqueous minimal mineral medium containing vitamin	GC/MS	Commercial soybean methyl esters Low sulphur diesel Blends of the above (B20, B40, B60 and B8)	Rapid loss initially of FAME from both biotic and abiotic microcosms Biological utilization rates for FAME increased with increasing carbon chain length and decreasing number of double bonds
Yassine *et al.* 2013b	Culture derived from wastewater activated sludge and gasoline- and triglyceride degrading cultures (acclimated to FAME and FAME/diesel blends	Aqueous minimal mineral medium containing vitamin	GC/MS CO_2 evolution Biomass production	Commercial soybean methyl esters Low sulphur diesel Blends of the above (B20, B40, B60 and B80)	Rapid loss initially of FAME from both biotic and abiotic microcosm 64% FAME mineralized within 7 days and 75% within 42 days C10-C12 alkanes metabolised faster in the presence of FAME
Fuller *et al.* 2013	Creek water	Creek water Oxygen	GC of polyaromatic hydrocarbons (PAH)	Tallow and canola oil FAME (10:90w/w) Commercial diesel B20 blend of the above	After 28 days, a higher proportion of the mass of polyaromatic hydrocarbons pyrene, fluoranthene, methylpyrenes, and C2-alkyl fluoranthenes and pyrenes were removed in the B20 blend than diesel alone
Horel & Schiewer 2014	Cultures isolated from soil and adapted to 1) fish oil alkyl ester 2) Syntroleum, or 3) diesel	Unsaturated sandy soil with NPK fertilizer added	CO_2 production; microbial enumeration; GC/MS	Fish oil alkyl ester (ester not specified) Diesel Syntroleum (synthetic oil)	Production of CO_2 from fish oil alkyl ester lagged behind that from diesel and the synthetic oil even using the inoculum adapted to fish oil alkyl ester; however, maximum CO_2 production in 28 days was similar for both diesel and fish oil alkyl ester (<20%)
Lisiecki *et al.* 2014	Microbial consortium isolated from a crude oil site and enriched on diesel as soil source of carbon; contained *Achromobacter*, *Alcaligenes*, *Citrobacter*, *Comamonadaceae*, *Sphingobacterium*, *Pseudomonas* and *Variovorax*	Mineral salts medium Oxygen, but microcosms were saturated, undisturbed and sealed	Carbon dioxide production GC of hydrocarbon fractions	Commercial rapeseed FAME Diesel (EN 590:2004) B10, B20, B30, B40, B50, B60, B70, B80, B90 blends of the above prepared in the laboratory	In the FAME and diesel microcosms, approximately 58% and 30% of FAME and diesel were mineralized to CO_2 within 578 days, respectively. The presence of FAME did not appear to enhance the rate or extent of diesel degradation. Rates of CO_2 production were as predicted by the ratio of FAME to diesel. Residual fractions of aromatic and aliphatic hydrocarbons were generally the same in the B10 through B80 microcosms.
Thomé *et al.* 2014	Clayey soil	Unsaturated soil with intermittent air flow; no amendments	Weight percent of residual hexane-extractable material	B20 blend of commercial soybean alkyl ester (ester not specified) with petroleum diesel	After 60 days, B20 reductions of up to 85% were observed for the aerated soils and 64% for the unaerated (possibly anoxic) control

The majority of the studies were conducted on either commercial or laboratory-produced FAME from rapeseed and/or soybean oils, with a few studies that used rapeseed or soybean ethyl esters. Isolated studies were also conducted on biodiesel produced from vegetable oil, animal fat, used cooking oil, castor oil and fish oil. Some authors did not specify the feedstock or the alkyl ester of the biodiesel. Only one study (Ginn et al 2012) considered the impact of additives on aerobic biodegradation. Studies were conducted on either pure fatty acid methyl/ ethyl esters, or blends with petroleum diesel (B2 to B80).

Several of the aerobic studies were conducted using Biological Oxygen Demand (BOD) procedures or modified procedures and used sewage sludge as the inoculum. These tests were monitored using either oxygen uptake and/or carbon dioxide production. Since sewage sludge contains very high biomass, which has generally been acclimated to fats and other lipids, these tests do not simulate conditions of natural attenuation in the environment, but do provide evidence of aerobic biodegradability and mineralisation by microorganisms. In most of these studies, 60 to 100% of soybean and rapeseed methyl or ethyl esters were reported mineralised to carbon dioxide within 21 to 28 days. One study that followed a standard BOD protocol (USEPA Method 405.1) reported mean BOD values (n = 6) for soybean and rapeseed methyl and ethyl esters and neat oils ranging from 1.53 to 1.74 mgl^{-1}, as compared to 0.40 mgl^{-1} for petroleum diesel as presented in **Table 6**. (Peterson & Moller 2005). The theoretical BOD cannot be calculated for comparison because the concentrations of the esters and neat oils in the tests were not given; however, chemical oxygen demand (COD), which can be used as a surrogate, was tested in parallel. As shown in **Table 6**, the ratios of BOD/COD values indicate that under the short term BOD test conditions, the fatty esters were biodegraded to a larger extent than were the neat oils and petroleum diesel.

Table 6: Experimental BOD and COD values

Test Material	BOD (mgl^{-1})	COD (mgl^{-1})	BOD/COD Ratio
Rapeseed ethyl ester	1.67	2.64	0.63
Rapeseed methyl ester	1.53	2.43	0.63
Neat Rapeseed oil	1.74	3.31	0.53
Soybean methyl ester	1.68	2.63	0.64
Neat soybean oil	1.57	2.70	0.58
Petroleum diesel	0.397	2.35	0.17

Data taken from Peterson & Moller 2005.
Mean Biological oxygen demand (BOD) (n = 6)
Mean Chemical oxygen demand (COD) (n = 3)

Other studies that were more representative of natural attenuation conditions used either environmental media (soil, groundwater or surface water) or bacterial cultures isolated from environmental media as sources of the inocula. The rates and extent of degradation observed in these studies were generally slower than in studies using sewage sludges, which generally contain a higher density and diversity of microorganisms than typical soil inocula as discussed in the introduction to Section 5.0. For example, Vauhkonen et al 2011 compared biodegradation of commercial rapeseed methyl esters and neat oil with either activated sludge or groundwater as the inoculum, as measured by oxygen uptake. Within 28 days, 60.8% RME and 65.8% neat rapeseed oil were biodegraded with the sludge inoculum, while only 19% rapeseed FAME and 9.9% neat rapeseed oil were biodegraded with groundwater as the inoculum. No further information was provided on the microorganisms present in the inocula.

To follow biodegradation of individual biodiesel components, some studies monitored degradation using GC to follow loss of the fatty acid esters. However, in some studies, loss of the fatty acid esters was noted to occur rapidly in both the biotic and abiotic control microcosms (e.g. Yassine et al 2013a and 2013b), indicating that hydrolysis of the esters was possibly a non-biological process or that the abiotic microcosms had a biotic component. Therefore, data from studies that only utilise GC to follow fatty acid esters are not sufficient to confirm that biodegradation has occurred unless the studies can demonstrate a difference between the biotic microcosms and abiotic controls, or provide a supportive measurement such as oxygen uptake or carbon dioxide production. For example, although Yassine et al 2013a and 2013b saw initial rapid loss in both the biotic microcosms and abiotic controls, the losses from the biotic microcosms were greater than those of the controls at later time points.

FAME from different feedstocks appear to be equally amenable to aerobic biodegradation based on the few studies that compared different FAME.

5.2.2. Degradation under anaerobic conditions (nitrate, sulphate, methanogenesis)

In the absence of molecular oxygen, other terminal electron acceptors can support anaerobic biodegradation processes. In soil and groundwater environments, these processes are typically reduction of nitrate, sulphate and/or metals (typically Fe(III) and Mn(IV)), as well as methanogenesis, which depends on carbon dioxide, acetic acid and certain other low molecular weight carbon compounds as electron acceptors. As summarised in **Table 7**, a number of studies in the literature provide evidence that biodegradation of FAME can be supported by nitrate reduction, sulphate reduction and methanogenesis; however, no laboratory studies on iron or manganese reduction were identified.

Table 7: Summary of Anaerobic Laboratory Studies of FAME and FAME-Diesel Blends

Reference	Source of Inoculum	Media	Measurement	FAME and Other Substrates	Reported Results
Stolz *et al.* 1995	Pond or lake water Top soil Culture grown on soybean FAME	Mineral salt medium Nitrate as electron acceptor	GC Methanol (by Draeger tubes) Nitrite	Soybean FAME	100% removal of FAME (as measured by GC) was observed in 14 days in anaerobic microcosms compared to about 50% in the uninoculated controls. 100% removal was observed in 7 days in parallel aerobic microcosms. The denitrifying bacterium H1 (*B. solanocearum*) was isolated using soybean FAME as the soil source of carbon
Cyplik *et al.* 2010	Microbial consortium isolated from crude oil site under aerobic conditions containing *Achromobacter, Alcaligenes, Citrobacter, Comamonadaceae, Sphingobacterium, Pseudomonas*, and *Variovorax*	Mineral salt medium, pH 7 Nitrate as electron acceptor	Nitrate and nitrite GC	Commercial rapeseed FAME Commercial diesel	20% removal of FAME vs 5% removal of diesel within 5 days, with 100% removal of FAME over the same period in parallel aerobic microcosms. Nitrate depletion and nitrite production was observed; the microbial community structure was similar when grown on FAME or diesel
Corseuil *et al.* 2011	Groundwater and sediments (unacclimated to FAME)	Groundwater and sediments Nitrate and sulphate as electron acceptors	GC Nitrate and sulphate	FAME from soybean oil and castor oil (from Paraná Institute of Technology)	Rate and extent of soybean oil FAME removal was greater than castor oil FAME; stearate (C18:0) disappeared more slowly from soybean FAME than the other C16 and C16 FAME; sequential depletion of nitrate followed by sulphate was observed with soybean oil FAME; however, only nitrate was depleted with castor oil FAME
Adair & Wilson 2009, 2010a and 2010b	Sediments from jet fuel release site Sediments from a biodiesel release site	Unspecified With or without sulphate as electron acceptor	Methane	Biodiesel (unspecified)	Conversion of 3mg biodiesel/L to methane was observed per day. The rate was similar in the presence and absence of sulphate
Aktas *et al.* 2010	Anaerobic cultures: 1) hydrocarbon degradation under sulphate and methanogenic conditions from contaminated aquifer sediments at natural gas field; 2) alkane-degrading methanogenic culture from contaminated aquifer sediments at natural gas field; 3) marine oil degrading sulphate reducing culture from sunken ship; 4) culture from seawater ballast tank; none, except possibly #4, were acclimated to FAME	Seawater or freshwater with CO_2/N_2 headspace; sulphate as electron acceptor followed by methanogenesis after sulphate depletion	Sulphate reduction Methane production GC	Soybean FAME	In the presence of FAME, sulphate was consumed by three of the inocula within one month with methane produced by two of the inocula; FAME was converted to free fatty acids ranging in chain length from C6 to 24 in the inoculated microcosms, while in the sterile controls only C16 to C24 FAME were observed; the detection of the shorter chain length fatty acids was consistent with β-oxidation; Sulphate reduction was observed

Borges *et al.* 2014	Lagoon sediments (unacclimated to FAME)	Mineral salt medium Sulphate as electron acceptor	Methane production Benzene by GC	Laboratory-produced FAME from soybean oil, castor oil and pork lard	Methane production was observed with about 40 days in all live microcosms and reached maximum methane production within 150 to 200 days; rates of methane production were similar among all three FAME (soybean, pork and castor). Short chain acids (acetate, propionate, formate, pyruvate and malonate) were detected with malonate the predominant acid in all three FAME microcosms
Sørensen *et al.* 2011	Water obtained in summer or winter from a light diesel storage tank diluted to ~10^6 cells/ml	Water from diesel tank Limited nitrate and sulphate	Methane production Biomass production	Commercial animal fat (pork lard) FAME Commercial fossil diesel	When water obtained during the summer was used as inocula, methane production was observed to occur for 13 days in the presence of FAME and blends of FAME with diesel (B5, B10, B20, B50), but not with diesel; a five-fold increase in biomass was also measured. No methane production was observed when water obtained during the winter was used as inocula.

Two studies investigated nitrate as the sole electron acceptor to support biodegradation of soybean oil FAME (Stolz et al 1995) and rapeseed oil FAME (Cyplik et al2010). Stolz et al 1995 reported 100% disappearance of soybean FAME within 14 days, while Cyplik et al 2010 reported 20% disappearance of rapeseed FAME within 5 days. Both studies reported that the disappearance of FAME occurred more slowly with nitrate as the electron acceptor than in parallel aerobic microcosms. In addition to disappearance of FAME in the presence of nitrate, both studies reported reduction of nitrate to nitrite during FAME degradation. Stolz et al 1995 also reported the formation of free methanol and an increase in biomass in inoculated microcosms, and were able to isolate a denitrifying bacterial culture that was capable of growth on FAME as the sole source of carbon. These results indicate that nitrate-reducing microorganisms are capable of biodegrading FAME and that the microorganisms are naturally present in fresh water.

Another study evaluated a mixture of nitrate and sulphate as electron acceptors for soybean and castor oil FAME using unacclimated soil and groundwater as the inoculum (Corseuil et al 2011). The rate of removal and extent of removal of soybean FAME was significantly faster and greater than castor FAME. The authors speculated that this difference might be due to the lower bioavailability of the more viscous castor FAME and its slower dissolution from the separate phase FAME into the water phase. Nitrate and sulphate showed sequential depletion over time in the soybean FAME microcosms relative to the abiotic control, but only nitrate was depleted in the presence of castor oil FAME. These data indicated that both nitrate and sulphate reduction was supporting biodegradation of soybean FAME microcosms, but only nitrate reduction supported degradation of the castor oil FAME. Another study by Aktas et al 2010 also demonstrated sulphate reduction in the presence of soybean FAME with naturally occurring microbial cultures that were isolated from hydrocarbon- contaminated aquifer sediments.

Several studies have confirmed biodegradation of FAME through methanogenesis with the production of methane (Aktas et al 2010; Borges et al 2014; Sorensen et al 2011; Adair and Wilson 2009, 2010a, and 2010b). Aktas et al 2010 demonstrated that when soybean FAME was incubated with naturally occurring microbial cultures isolated from hydrocarbon-contaminated aquifer sediments, all produced methane at concentrations of greater than 20 milliMolar (mM) within one month. However, insufficient information was provided to determine the extent of FAME degradation to methane. GC analysis confirmed that the soybean FAME had been converted to free fatty acids with chain lengths from C6 to C24. Most of the shorter chain length fatty acids were even-numbered and indicated that degradation under methanogenic conditions was likely occurring through β-oxidation.

FAME from animal fat and castor oil has also been shown to be amenable to biodegradation through methanogenesis. Sorensen et al 2011 incubated animal fat FAME, diesel and blends of FAME and diesel (B5, B20, and B50) with water obtained from a diesel storage tank. Methane was produced from day 0 to day 13 in microcosms containing B100, B50, B20, B10 and B5, while methane was not produced in the diesel-only microcosms. Biomass also increased over the course of the incubation. Borges et al (2014) compared the biodegradation of soybean oil, pork lard and castor bean oil FAME with synthetic groundwater and sediment from a lagoon. Methane production was observed within about 40 days in all microcosms and reached maximum methane production within 150 to 200 days. The rates of methane production were similar among all three FAME (soybean, pork lard and castor). Low molecular weight fatty acids (acetate, propionate, formate, pyruvate and malonate) were detected with malonate being the predominant acid with all three FAME.

Adair & Wilson (2009, 2010a & 2010b) have reported rates of methane production of 3 mgl^{-1} methane per day from the biodegradation of biodiesel (source oil not specified) at 1000 mgl^{-1}, in microcosms constructed with aquifer sediments from a jet fuel release site and a commercial soybean FAME release site (which is discussed in more detail later in this review). Although the longevity of methane production in biodiesel degradation is not known, methane generation may, like ethanol-release sites, be a significant risk driver at large biodiesel release sites (Adair and Wilson 2010a).

No laboratory studies investigating FAME degradation through Fe(III) or Mn(IV) reduction have been identified; however, as discussed later in this review, biodegradation of FAME by iron oxide reduction has been observed in the field (Toso 2010 & 2012).

Although fewer laboratory studies have been conducted under anaerobic conditions than aerobic conditions, the studies all confirm that FAME biodegradation can occur through nitrate reduction, sulphate reduction and methanogenesis.

5.2.3. Effect of Additives on Biodegradation

Very few studies have reported on the presence of additives in the biodiesel used in biodegradation testing and even fewer have reported on whether the presence of an additive had an effect on biodegradation. As discussed in more detail later, Toso (2010 & 2012) reported on a large-scale release from an aboveground tank in Minnesota, USA, of FAME containing the anti-oxidant butylhydroquinone (TBHQ) at a concentration of 250 mgl^{-1}. Evidence of both aerobic and anaerobic biodegradation of the released FAME was observed and indicated that the presence of TBHQ did not prevent biodegradation. However, whether TBHQ enhanced, inhibited, or had no effect on biodegradation could not be determined from available information.

Ginn et al 2012 investigated the effect of an anti-oxidant additive (BioExtend) and an unspecified biocide on the aerobic degradation of FAME from soybean oil and animal fat. The composition of BioExtend was not reported in the paper; however, BioExtend is an antioxidant product from Eastman that contains TBHQ and a metal chelating agent. The microcosms were constructed with a soil inoculum and either without additives, with the anti-oxidant additive (BioExtend), or with the anti-oxidant and an unspecified biocide. No significant impacts on carbon dioxide production over 28 to 30 days from FAME (or parallel microcosms constructed with diesel or a B20 blend of either soybean or animal fat) were observed in the presence of either additive.

Research into the biodegradation of compounds related to FAME, such as vegetable oil precursors, has shown that biodegradation rates may be increased in the presence of antioxidant. For example, Salam et al 2012 found that the antioxidant butylated hydroxytoluene (BHT) increased vegetable oil (linoleate triglyceride) mineralisation from 41 to 61% after 19 weeks of incubation, possibly due to BHT inhibiting the formation of less bioavailable polymers (significant polymerisation was observed in microcosms without BHT).

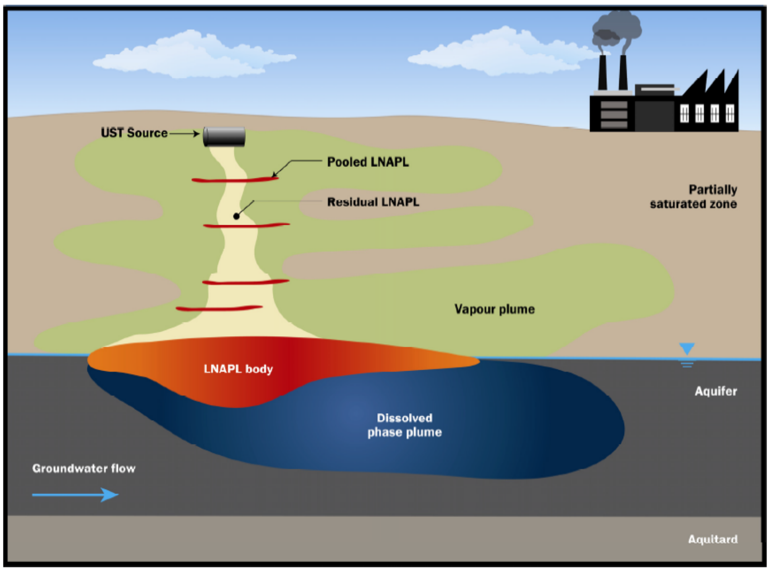
6. PARTITIONING BEHAVIOUR OF FAME IN SUBSURFACE

In 2008, the Energy Institute concluded that little research had been done on the fate and transport of alkyl ester based biodiesel. However, given the similarity in physical properties between alkyl ester based biodiesel and conventional diesel, they anticipated that the likely fate and behaviour of FAME-containing biodiesel would be comparable to conventional diesel. With similar viscosity, density and hydrophobicity (see **Table 2**), B100 would form a Light Non-Aqueous Phase Liquid (LNAPL) that would behave in a similar manner to diesel LNAPL (Ginn et al 2012). Solubility and partition coefficient data indicates that FAME would be strongly sorbed to soil organic matter and relatively immobile in groundwater (Energy Institute 2008). Ramos et al (2012) summarise that biodiesel blend releases are not readily miscible in groundwater and behave as a fixed, decaying, yet long lived source with relatively small region of influence compared to more mobile biofuels such as ethanol.

The fate and behaviour of petroleum hydrocarbons in the subsurface is well understood and is influenced by the nature of the initial release, the physical –chemical properties of the hydrocarbons and the site specific geological, hydrogeological and geochemical characteristics of the receiving environment. Detailed guidance on the processes of concern and the development of light non aqueous phase liquids (LNAPL) specific models is available from a number of sources (ITRC 2009 & CL:AIRE 2014).

Elements of an LNAPL conceptual site model (Figure 4) include an understanding of contaminant fate and transport in the unsaturated zone, the interaction of LNAPL with groundwater at the saturated zone interface and the subsequent migration and fate of either LNAPL or the dissolved phase in groundwater according to the prevailing hydrogeological regime.

Figure 4: Schematic of LNAPL Conceptual Model (after CL:AIRE 2014)



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6.1. FAME BEHAVIOUR IN UNSATURATED ZONE

As part of the 2015 California EPA Multimedia Evaluation of Biodiesel, Ginn et al 2012 conducted a series of small scale experiments in two-dimensional sandboxes to visualise the rates of biodiesel LNAPL infiltration, redistribution and lens formation on the water table relative to that of ultra- low sulphur diesel (ULSD). Experiments involved 20cm vertical infiltration through unsaturated sand and experiments were performed in triplicate for animal fat (AF) and soybean (Soy) derived biodiesel at B100 and B20 blends. Each fuel had been treated with the antioxidant BioExtend (which contains TBHQ), as per manufacturer's instructions. Each experiment was run to effective steady state lens formation. The experiments found that the behaviour of Soy B100, Soy B20 and animal fat B20 LNAPL was similar to that of ULSD. In the case of AF B100 there was a noticeable difference in the amount of residual biodiesel NAPL retained in the unsaturated zone and in the NAPL lens geometry. This was attributed to the physical properties including higher viscosity and interfacial tension of animal fat biodiesel relative to ULSD. The report concluded that these differences may become more pronounced at temperatures below 20°C.

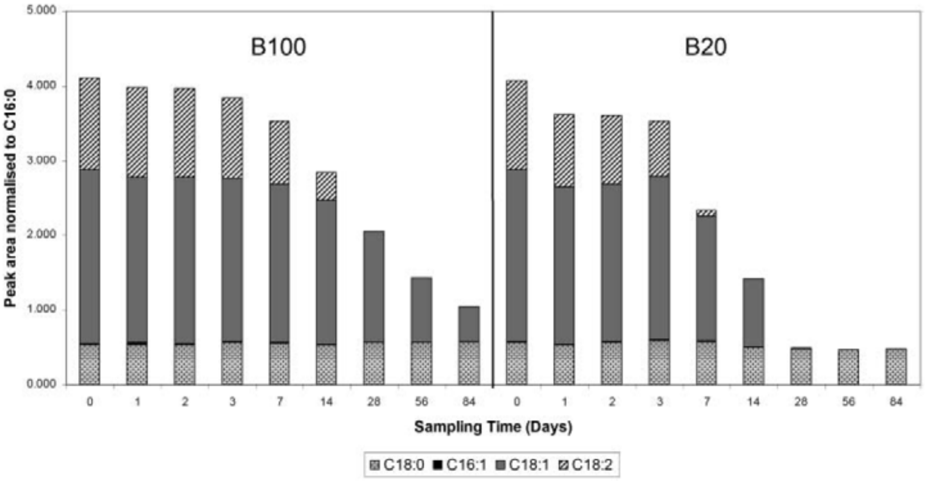
Field studies that validate the above have not been published. Courseil and colleagues have undertaken controlled release experiments of B100 and B20 release at a field site (Chiaranda and Courseil 2010, and Ramos et al 2012). However, the methodology involved the excavation of the subsurface down to the water table and emplacement at the water table rather than via infiltration from the subsurface. Toso (2010) reported on the investigation of a large- scale subsurface release of approximately 110m^3 of soy based B100 (with TBHQ antioxidant (250ppm)) through corrosion holes in the base of an above ground storage tank. The release was adjacent to a river in an area of generally sandy soils and fill. Depth to water was approximately 2 to 3 meters below ground surface. LNAPL was recovered from trenches around the release and 0.57m of LNAPL was observed in a monitoring well near the tank though this LNAPL was transient and did not reappear following initial removal via bailing. It was noted by Toso that the behaviour of B100 as a LNAPL seemed to match that reported in the California Multimedia Evaluation of Biodiesel sandbox study.

6.2. BEHAVIOUR OF FAME IN LNAPL

In the subsurface the FAME component of LNAPL will partition into the environment according to the properties of the FAME, other LNAPL components, and the subsurface. A number of studies have examined compositional changes in FAME within LNAPL. These studies have been undertaken primarily in the context of surface waters or fuel stability but are also considered relevant to the subsurface.

Fuller et al 2013 investigated whether compositional changes in FAME and biodiesel LNAPL could be used for the forensic assessment of biodiesel spills. A stable layer of FAME or biodiesel was maintained over an aqueous phase in microcosms and compositional changes in FAME, sterols and hydrocarbons were observed over time. The LNAPLs tested comprised 100% FAME (produced from a commercial mixture of tallow and canola oil (10:90 w/w)), petroleum diesel and a B20 blend of these components (the presence or absence of antioxidants or other additives was not reported). The experiment was designed to mimic a surface water body. Microcosms were incubated in a greenhouse under varying light levels and with temperature varying from 24.5°C to 26.7°C. Samples were collected over a period of 84 days. Analysis focused only on the LNAPL phase and not on water composition. In terms of overall mass changes, a gradual reduction in the B20 and diesel mass was observed, with 33% to 38% of the initial mass remaining after 84 days, while 90% of the mass of B100 remained after the same period. For individual FAME compounds, the results indicated that for both the B100 and B20 microcosms, major changes were evident in individual FAME after 7 days and that the unsaturated FAME were the first to decrease in concentration, with polyunsaturated FAMEs degraded before monounsaturated FAMEs (**Figure 5**). The concentration of saturated FAMEs did not change appreciably during the experiment. The removal of individual FAME compounds general occurred faster for B20 compared to B100. A number of oxidation products were observed including 9-oxononaoic acid methyl ester and cis-3-octyl-oxiraneoctanoic acid methyl ester. Both are associated with oxidation of unsaturated C18 FAME. Photo-oxidation and biodegradation were considered the main weathering mechanisms for biodiesel and biodiesel blends.

Figure 5: Fatty acid methyl esters (FAME) profile changes of B100 and B20 over the course of the weathering experiment. The peak area of the FAMEs are normalised to C16:0 (C14:0, C16:0 and C17:0 not shown) (Fuller et al 2013)



In a similar study Khoury et al 2011 examined the degradation/photolysis of 3 commercial FAME- based biodiesels and one FAME synthesised from Canola oil. Samples were exposed to ambient sunlight and seawater over a period of 71 days. In a separate experiment an assessment of the role of photolysis was made by comparing samples stored in the dark with others left in light. Unsaturated FAME were preferentially photo-oxidised and disappeared rapidly. Saturated FAME including C14:0, C16:0 and C18:0 were the most persistent.

Yang et al 2013 also studied changes in the mass and chemical composition of pure soybean FAME, canola oil FAME (commercial source with antioxidants), ultra- low sulfur diesel (ULSD) and B5 (canola), B20 (soy) and B20 (canola) blends of these components. Tests were carried out in the dark at ambient temperatures (~22°C) with air exposure for 190 days. The mass loss curves indicated losses for pure diesel (B0) and the B5/B20 blends but no losses in the B100. The loss was attributed to evaporation of the diesel components. Yang et al 2013 noted that the mass loss via evaporation is significantly lower for biodiesel than conventional diesel because of the higher boiling point of the biodiesel components. Losses due to microbial activity and chemical degradation were negligible and the authors implied that this was possibly due to unidentified antioxidants in the commercial biodiesel used.

The studies by Yang et al 2013 and Khoury et al 2011 provide insight into compositional changes that may occur in FAME and biodiesel LNAPL in the presence and absence of air, sunlight and water. In the absence of water, physical removal processes such as volatilisation are only significant for the more volatile petroleum diesel fraction. However, in the presence of sunlight and/or water significant photo-oxidation and biodegradation of the FAME component is also possible.

6.3. DISSOLUTION OF FAME IN GROUNDWATER

Research papers on the in-situ dissolution of FAME in groundwater were not identified during this review. However a number of studies did examine the partitioning behaviour in aqueous solutions and these are summarised below.

The aqueous solubility of commercial FAME biodiesels, ultra-low sulphur diesel (ULSD) and FAME biodiesel- ULSD mixtures was examined by Hollebone et al 2008 as part of a wider study into aquatic toxicity. The equilibrium water accommodated fraction (WAF) concentration in freshwater was determined for the biodiesels and B20 and B5 blends. WAFs were generated by adding 10g of test oil to 1 litre of water and stirring at a constant rate for 16 hours, with a 30 minute rest period prior to sampling and analysis by gas chromatography with flame ionisation detection (GC-FID). For ULSD the WAF concentration varied from between 23 and 45 mgl^{-1} with the soluble fraction consisting of BTEX and alkylated benzenes. For the biodiesels WAF concentrations were variable and ranged from 13 mgl^{-1} to 105 mgl^{-1} for soybean FAME, and up to 101 mgl^{-1} for fish waste FAME. The authors concluded that the solubility of FAME is highly variable even for the same feedstock type. The WAF of B5 and B20 biodiesels varied from 21-29 mgl^{-1} and the authors noted no measurable co- solvent effects between FAME and diesel mixtures. The components in the FAME WAF were not quantified but were considered heavier than those seen in the diesel and appeared to be “oxygenated hydrocarbons”.

Yassine et al 2012 studied the partitioning behaviour of diesel, soybean FAME and soybean FAME- diesel blends (B20, B40, B60 and B80) in water at a variety of oil loads. Water accommodated fractions (WAFs) were prepared by adding the oil to a series of 500ml flasks at oil:water loading ratios ranging from 1:1 to 1:1000 (V/V). The flasks were capped and stirred in the dark for 24 hours maintaining a constant vortex depth of 20%-30% of the total depth of the water column, (the temperature of the flasks was not specified). After equilibration, the samples were transferred to separating funnels and allowed to settle for 2 hours (in dark) prior to sample collection and analysis. For the lowest loading ratio (1:1000) the WAF sample was filtered through 3mm deactivated borosilicate glass beads to remove any particulate oil present in the WAF prior to analysis. The FAME analysis was compared for each biodiesel and loading ratio, and the respective aqueous solubility calculated using Raoult’s law. C16:0, C18:1, C18:2 and C18:3 methyl ester concentrations at the 1:1000 loading ratio were reported to be in fair agreement with reported aqueous solubilities for all the blends. In a method analogous to that used by Hollebone et al 2008, Yassine et al 2012 also measured the total organic carbon (TOC) content of the WAF. This was found to increase linearly according to the oil loading with a WAF total organic carbon (TOC) of between 50.7 mgl^{-1} and 95.4 mgl^{-1} for diesel and soybean FAME respectively at the 1:1 loading ratio. At lower oil loads, the trend was reversed with WAF TOC of between 3.74 mgl^{-1} for diesel and 1.4 mgl^{-1} for soybean FAME, respectively, at the 1:1000 loading ratio. Analysis of the WAFs by gas chromatography with detection by mass spectrometry (GC-MS) indicated the presence of free-phase methanol, n-hexanal, n-butyl acetate, diethylene glycol monobutyl ether and diethylene glycol monobutyl ether acetate and other peaks that were reportedly not present in the parent oils. These products were attributed to the hydrolysis of FAMEs into free fatty acids and methanol. Further analysis indicated the loss of 5-10% of the FAME profile after the 24 hour equilibration period and in addition insoluble fine deposits assumed to be biodiesel oxidative polymerisation products were also noted within the vessel. The findings suggested rapid autoxidation of biodiesel in the conditions studied.

No field based measurements of FAME solubility in groundwater were identified for this review.

7. FIELD STUDIES OF BIODEGRADATION OF FAME IN THE SUBSURFACE

The laboratory studies discussed in the previous sections provide evidence that biodegradation can occur under a range of redox conditions. However, field data is required to understand the significance of these processes for FAME biodegradation in soil and groundwater. A controlled release of soybean FAME biodiesel and B20 was implemented in Brazil in June 2008 and continues to be monitored. A large-scale accidental release of soybean FAME biodiesel from an AST occurred in 2007 in the United States in Minnesota. The findings from these field studies are discussed below.

7.1. CONTROLLED RELEASE OF FAME AND BIODIESEL (B20)

As indicated earlier, Chiaranda (2011) and Ramos et al (2012) have reported results from controlled releases of soybean oil FAME (B100) and a B20 blend of soybean oil FAME and petroleum diesel at a field site at the Experimental Farm of Ressacada in Florianopólis, Brazil. The experiments commenced in June 2008 initially as natural attenuation studies (Chiaranda 2011) but some of the plots were subsequently amended with ammonium acetate to stimulate biodegradation (Ramos et al 2011; Ramos et al 2012). At the commencement of studies, approximately 100 litres of each was released into each of two one-meter square plots that had been excavated 1.6 metres down to the water table (Chiaranda & Corseuil 2010; Chiaranda 2011). Both plots were covered to limit rainfall into the excavations. The geology is generally a fine sand with some silt and clay. Groundwater flow velocity is slow and has been estimated at 6 m per year. The site is located in Brazil and reported groundwater temperatures range from 22ºC in the winter to 26°C in the summer. The groundwater pH was 4; although not noted in the report, very acidic pH is common in Brazil.

Groundwater quality was measured at the source and approximately 2m downgradient over a period of 29 months. Each of the plots slowly changed from aerobic to anaerobic conditions with oxidation-reduction potentials in groundwater slowly transitioning from +430 millivolts (mV) to -20 mV. In the B100 plot, acetate concentrations peaked at about 120 mgl^{-1} between 1.5 and 2 years after the release. Changes indicative of a range of redox processes were apparent at the source and downgradient with alkalinity increasing from 50 mgl^{-1} to ~250 mgl^{-1}, ferrous iron peaking at about 100 mgl^{-1} between 1.5 and 2 years after the release, sulphate reducing from 25 mgl^{-1} to below detection over 12 months and methane concentrations in groundwater increasing from below the detection limit to a maximum of 30 mgl^{-1}, which is in excess of the solubility limit for methane in water (25 to 28 mgl^{-1} at the reported groundwater temperatures).

Analysis of the dominant TEAP over time indicated a transition from iron-reducing to methanogenic conditions. In the B20 plot changes in TEAP were similar though less consistent than those in the B100 plot: dissolved oxygen was already depleted; nitrate was present at initial concentrations varying from approximately 40 to 80mgl^{-1} but was rapidly reduced to below detection in three months. Iron reduction was less marked with concentrations peaking at approximately 20 mgl^{-1} and sulphate increasing or stable. Methane peaked at about 30 mgl^{-1} between 1.5 and 2 years after the release. Concentrations of acetate remained on or near detection limits for the whole experiment. For the B20 release, chemical analysis was also undertaken of benzene, toluene, ethylbenzene and xylenes (BTEX) and total hydrocarbons. BTEX concentrations were either stable, or more typically increased over the duration of the project. It was, however, not possible to determine whether biodegradation rates of BTEX were reduced in the presence of FAME due to the absence of a diesel-only control. A follow up paper by Ramos et al 2014 focused solely on results from the plot amended with ammonium acetate.

7.2. LARGE-SCALE ENVIRONMENTAL RELEASE OF FAME

Although FAME and FAME/ diesel blends have been in use since the late 1990s, only one accidental release of FAME into the environment has been reported in the literature (Toso, 2010 & 2012). This release occurred in the United States in the State of Minnesota in August 2007, as described earlier. Although individual FAME compounds were not detected in groundwater (possibly due to detection limits above the aqueous solubilities), elevated concentrations of Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC) were detected at concentrations as high as 2,390 mgl^{-1} and 2,340 mgl^{-1}, respectively. Short chain fatty acids including acetate and butyrate, which are products of biological beta-oxidation of FAME, were detected at 2,094 mgl^{-1} at one well and accounted for 85% of DOC at that location. This indirect evidence for biodegradation was further supported by the detection of high dissolved inorganic carbon, high methane and high ferrous iron in groundwater. Soil gas measurements also detected high methane (up to 67%) and carbon dioxide (up to 34%) with complete depletion of oxygen at these same locations. The rapid development of anaerobic conditions in groundwater was reported. Although no data on Oxidation Reduction Potential (ORP) or dissolved oxygen were presented, the high concentrations of methane and ferrous iron, both of which form under strongly anaerobic conditions, provide good evidence that anaerobic conditions developed.

Taken together, these data provide strong evidence that FAME was attenuated by biodegradation in the subsurface. The naturally occurring microbial population, which likely had no previous exposure to FAME, was capable of metabolising FAME through multiple TEAP including aerobic, iron reduction and methanogenesis.

Ultimately, a full understanding of the behaviour and natural attenuation of FAME in soil and groundwater will only come from observations of FAME at multiple release sites, as was done in the past for gasoline and other petroleum products (Rice et al 1995; Mace et al 1997; Newell et al 1989) and more recently for ethanol (Ruiz-Aguilar et al 2002; Mackay et al 2006; Morgan et al 2014). In this regard, further data is also required on the behaviour of biodiesel and biodiesel/ petroleum diesel blend releases in different geologies, hydrogeological regimes and climates.

8. EFFECT OF FAME ON PETROLEUM HYDROCARBONS

FAME may interact with hydrocarbons in the subsurface to modify their physical and natural attenuation behaviour. In this review the following modes of interaction are considered:

- FAME as an agent for the mobilisation of residual or sorbed hydrocarbons in the subsurface as a results of a biodiesel infiltration onto previously impacted ground;
- effects of FAME on the stability and behaviour of diesel LNAPL in biodiesel blends;
- effects of FAME on the solubility and partitioning of individual hydrocarbon components from biodiesel mixtures to the water environment; and
- influence of FAME on the biodegradation of petroleum hydrocarbons.

8.1. POTENTIAL MOBILISATION OF HYDROCARBONS

The potential use of FAME to mobilise hydrocarbons in the subsurface has been the subject of research, and previous reviews have highlighted a number of papers where FAME have been studied in the context of mobilising crude oil or polycyclic aromatic hydrocarbons (PAH) from soils (Energy Institute 2008 and ITRC 2011).

These works included a study by Miller and Mudge (1997) that evaluated the application of rapeseed oil FAME on the weathering and removal of crude oil within artificial sand columns. The addition of FAME led to greater recovery of oil from the sediment when applied to relatively unweathered crude oil. This enhancement was the result of the crude oil dissolving within the more mobile FAME. In a follow up study, Pereria & Mudge 2004 evaluated the use of FAME produced from rapeseed, soybean and cooking oil at the laboratory scale in microcosms containing crude oil to which FAME was added at ratios of 1:2, 1:1 and 2:1 FAME: crude oil. Crude oil removal rates of up to 96% were reported for the rapeseed oil FAME compared to a value of 15% in a control using water only. In the mesocosm experiments a ratio 1:1 of soybean FAME to crude oil removed 80% of the oil in cobbles and fine sands, 50% in coarse sand and 30% in gravel. Taylor and Jones (2001) examined the addition of nutrients, diesel and FAME on the biodegradation of coal tar in field and laboratory experiments. Increased degradation of naphthalene following application of FAME was attributed to tar solubilisation and dispersion leading to an increase in PAH bioavailability.

More recently, Gong et al 2010 studied the use of FAME on the solubilisation of PAH from soils from a former manufactured gas plant (MGP) site and two soil samples artificially spiked with PAH. The study undertook a comparison between the use of a commercial FAME (manufactured from waste oil), a laboratory-synthesised soybean oil FAME, soybean oil, methanol and a range of other surfactants. For the FAMEs and oils, 5ml was added to 5g of soil. The mixture was sealed in a glass bottle and shaken for 16 hours on an orbital shaker at 160rpm at 30°C, whereupon it was centrifuged to separate the eluting agent and soil. Total PAH removal of between 35% (commercial FAME) and 46% (soybean FAME) was reported and this removal was greater than that observed for the soybean oil, the methanol or the commercial surfactants.

Each of the above case studies illustrates the potential for FAME at high concentrations to mobilise previously spilt and residual hydrocarbons in the subsurface, which could be relevant if a FAME spillage was to occur at a historically impacted site. However, to date no field or case study examples of such behaviour appear to have been reported. Due to the likely site- specific nature of any such effects, further study in this area is recommended.

8.2. EFFECT OF FAME ON STABILITY AND BEHAVIOUR OF DIESEL NAPL

Most of the work on the effect of FAME on the behaviour of diesel LNAPL has been undertaken in the context of the fate of biodiesel in the marine surface water environment.

DeMello et al 2007 observed that in dispersion experiments simulating turbulent mixing FAME blends of B20 or greater in seawater, FAME (unspecified source oil) appeared to stabilise oil droplets in the water phase by decreasing the oil water surface tension and, therefore, reducing oil droplet aggregation. The authors concluded that FAME may influence the transport, weathering rate and ecological impact of spilled biodiesel and suggested FAMEs may enhance dissolution rates of conventional hydrocarbons, and by extension increase their potential for biodegradation.

Similarly, Hollebone et al 2008, reported that under high energy conditions (rotating flask), commercial FAME biodiesels and B20 blends produced from soy oil, canola oil, waste fry tallow and fish waste oil were dispersed much more readily in freshwater than petroleum diesel fuel tested under the same conditions.

Both the above studies were undertaken in turbulent conditions unlikely to be representative of conditions in groundwater. Hollebone et al 2008 noted that under low energy conditions the biodiesels tested had very similar behaviour to the petroleum diesel.

Yang et al (2015) studied the photolysis behaviour of FAME and FAME-petroleum diesel blends in surface water using FAME prepared from soybean, canola and animal fat. Reduced photolysis of some heavy alkanes and PAHs was reported due to the stabilisation by FAME of small oil droplets formed by agitation. This effect, while decreasing photolysis rates, would increase the oil/ water contact area and so likely increase the rate of biodegradation.

Overall, FAME at concentrations of B20 and higher appears to increase the amenability of certain hydrocarbons in NAPL to photoxidation by sunlight. Under subsurface conditions the presence of FAME at ≥B20 may lead to increased NAPL/ water mixing, thereby increasing the surface area for biodegradation. No studies were found reporting the effect on diesel properties at lower FAME blending ratios. In addition, no field or case studies were identified addressing the in-situ effect of FAME on biodiesel NAPL stability in groundwater.

8.3. EFFECT OF FAME ON PARTITIONING OF HYDROCARBONS

Coupled with (and difficult to separate from) physical effects on the stability of NAPL may be the influence of FAME on the volatilization and dissolution of individual hydrocarbons. A number of studies have reported on such aspects.

In respect of volatilisation, in separate experiments undertaken as part of the studies referred to above, diesel hydrocarbons were observed to evaporate at a similar rate for both diesel and biodiesel/ diesel mixtures (DeMello et al 2007). This behaviour was corroborated by additional calculations of activity coefficients in simulated diesel and biodiesel/ diesel mixtures and De Mello et al 2007 concluded that FAME will not affect the rate of volatilization of diesel components.

This observation was later confirmed by Yang et al 2013 studying the weathering of FAME, diesel and B5 and B20 blends in the open air under ambient conditions over a period of 190 days. To evaluate the contribution of FAMEs to the evaporative mass loss of blend mixtures, they calculated the theoretical mass loss curves for the B5 and B20 blends (by linearly scaling the pure diesel loss curve by the blend ratio) and compared these curves with the actual losses. From the results of this analysis, Yang et al 2013 inferred that FAMEs did not affect the evaporative losses of diesel hydrocarbons.

With regard to partitioning and mass transfer to the aqueous phase, Hollebone et al 2008 measured the WAF of B5 and B20 blends in comparison to that of diesel. The WAF of the blends were not significantly different from the diesel product and it was concluded that there were no measurable co-solvent effects between biodiesel and petroleum diesel in the blends.

Chen et al 2008 studied the oil-water partitioning of eight indicator PAH, with the oil phase comprising FAME derived from waste edible oil and B1, B5 and B20 blends of this FAME with petroleum diesel. A batch equilibration technique was used whereby oil and water (1:10 ratio) were mixed using horizontal agitation for a period of 24 hours before analysis of the aqueous phase. It was found that oil- water partition coefficient values (K_{fw}) for most PAH lay near the ideal line, such that the partitioning of PAHs between biodiesel and water could be described using Raoult’s Law (errors within a factor of two). The authors note that in the subsurface PAH solubility will depend on many other factors, including sorption to the aquifer matrix and the presence of potential surfactants, emulsifiers or cosolvents.

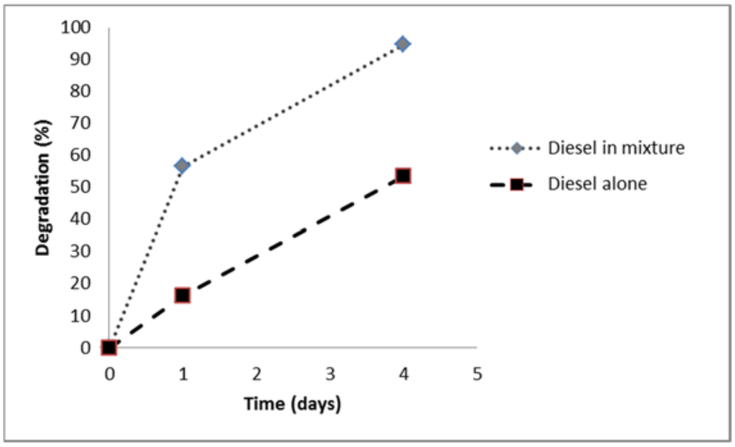
In the experiment referred to earlier, Yassine et al. (2012) also studied the partitioning of C10-C20 n-alkanes and four monoaromatic compounds in petroleum diesel/ soybean FAME mixtures. Concentrations of n-alkanes in WAFs from diesel and B20, B40, B60 and B80 blends progressively increased with the FAME content of the blend, with an increase in both the dissolved and colloidally accommodated n-alkane load reported. Micelle formation was considered to be enhanced in the presence of FAME and FAME autoxidation byproducts (hexanal, n-butyl acetate, diethylene glycol, monobutyl ether, and diethylene glycol monobutyl ether acetate were positively identified). In contrast, concentrations of aromatic compounds were found to closely obey Raoult’s Law at all oil loads. Overall Yassine et al. concluded that a >B20 blend spill would be easier to treat by bioremediation than a petroleum spill due to enhanced dissolution of aliphatic compounds and the presence of colloidal micro- emulsions increasing the oil/ water contact area. It was noted, however, that the partitioning behaviour of biodiesels will vary according to their formulation (e.g. feedstock source, additives and methods of manufacture).

8.4. EFFECT OF FAME ON BIODEGRADATION OF PETROLEUM HYDROCARBONS

Since FAME is commonly sold blended with petroleum diesel, it is important to consider the effect of diesel on FAME biodegradation, and FAME on diesel biodegradation. Published laboratory studies that address these effects are summarised in **Tables 5 and 6,** for aerobic and anaerobic conditions, respectively.

Zhang et al 1998 compared the rate of biodegradation of diesel (Phillips 2-D reference diesel) under aerobic conditions in the presence and absence of rapeseed ethyl ester, using both mineralisation to carbon dioxide and gas chromatography (GC). This paper was one of the first to report that fatty acid esters appear to enhance the biodegradation of diesel. The tests were conducted on rapeseed ethyl ester, diesel and B80, B60, B20 blends of these components. Tests were run in mineral salts media amended with yeast extract and amino acids, and inoculated with organic-rich soil, aerated activated sewage mixed liquor and raw domestic sewage water. The mixtures were then monitored for evolution for carbon dioxide production. The percent of added carbon mineralised to carbon dioxide over 28 days increased with increasing portion of rapeseed ethyl ester in the mixture. In a second experiment using a 50:50 diesel /rapeseed ethyl ester mixture (B50), 56.5% of diesel in the mixture with rapeseed ethyl ester and 16.3% of diesel alone disappeared as measured by GC by day 1. By day 4, 94.8% of the diesel disappeared as measured by GC when present in the B50 mixture, while only 53.5% of the pure diesel (B0) in a control had disappeared, as shown in **Figure 6**. The authors concluded based on the disappearance of diesel from the chromatograms that the alkanes of diesel and the fatty acid ethyl esters were degraded at similar rates and estimated that the maximum removal rates as determined by CO_2 evolution were 25 $mgl^{-1}day^{-1}$ for rapeseed ethyl ester and 12.5 mgl^{-1} for diesel.

Figure 6: Degradation of diesel in biodiesel / diesel mixture compared to biodegradation of diesel alone (starting concentration 10 mgl^{-1}) (redrawn from Zhang et al 1998)



Taylor & Jones (2001) examined the addition of nutrients, diesel and FAME on the biodegradation of coal tar in field and laboratory experiments. The degradation of naphthalene in coal tar over 55 days was shown to increase by 85% in laboratory experiments and 52% in field experiments when FAME was applied with nitrate and phosphate, whereas a decrease in degradation was observed when diesel was used.

Pasqualino et al 2006 reported that FAME derived from waste cooking oil increased both the rate and extent of mineralisation of diesel and gasoline in aerobic microcosms inoculated with activated sludge.

DeMello et al 2007 investigated the effect of FAME on the biodegradation of diesel in aerobic seawater microcosms. Diesel and FAME (B100, source oil not specified) were obtained from commercial sources and mixtures of diesel and FAME were prepared (B8 and B25). Several commercially available B20 mixtures were also obtained for comparison. [The authors noted that the proportion of diesel in these B20 commercial mixtures exhibited “extreme variability”, with one sample having almost no diesel present, possibly the result of a blending problem.] Microcosms were constructed using sea water amended with nitrogen and phosphorus and either B8, B25, B100 or petroleum diesel (to approximately 0.1125 mgl^{-1}). Abiotic controls were constructed in parallel. The microcosms were incubated on a shaker table in the dark under aerobic conditions and analysed by gas chromatography for n-alkanes, branched-chain alkanes, individual FAME, bulk FAME and bulk diesel. Biodegradation of diesel followed a typical pattern of loss of n-alkanes with relative increased proportions of branched-chain alkanes (farnasene, norpristane, pristine and phytane) and the unresolved compound mixture (UCM). A similar pattern of losses was observed in the B8 and B25 mixtures, along with loss of FAME. The n-alkanes were observed to disappear slightly slower initially in B8 and B25 than in the pure diesel; however, this effect was small and could only be shown using the n-C18 to phytane ratio. Using two-dimensional gas chromatography, no differences were apparent in the composition of the UCM in the presence or absence of FAME; however, this technique is relatively crude compared to techniques such as mass spectrometry with selective ion monitoring for resolving these residual compounds. Overall, the conclusions from this study were that FAME has little effect on the biodegradation of straight-chain and branched-chain alkyl components of diesel under aerobic conditions.

Mariano et al. 2008 used a respirometer to assess the biodegradability of (a) commercial B2 biodiesel (source oil not specified), (b) castor oil FAAE (ester type unspecified), (c) commercial diesel and B5, B20 biodiesel blends of (b) and (c). Test substrates were incubated under aerobic conditions with either contaminated river water or fuel-contaminated soil from a petrol station. Carbon dioxide production from castor oil FAAE and castor oil FAAE /diesel blends was much higher than from diesel, while the carbon dioxide production from diesel was similar to the production from the commercial B2 biodiesel. The carbon dioxide production from the B20 blend was essentially the same as predicted from the production observed from pure diesel and pure FAAE. Thus, the blending of FAAE and diesel did not show enhancement of carbon dioxide production.

Owsianiak et al 2008 presented limited data that addition of commercial rapeseed FAME at 10% to diesel (B10) inhibited biodegradation of the diesel fraction under aerobic conditions by approximately 10%, but at 30% FAME (B30) and higher may have enhanced biodegradation of the diesel fraction by approximately 5%. The microcosms were constructed in a mineral salts medium using a microbial consortium isolated from a crude oil site, as described earlier. Mass removal of FAME and diesel was determined by GC over seven days. Biomass development within the microcosms increased with increasing FAME content. Biodegradation of diesel appeared to increase with biodiesel content.

While investigating FAME biodegradation under anoxic/anaerobic conditions, Corseuil et al 2011 also looked into the effect of FAME on the biodegradation of benzene and toluene. Microcosms were constructed using groundwater and sediment, purged with nitrogen to remove oxygen, and amended with either benzene and toluene (2.9 mgl^{-1} and 0.8 mgl^{-1}, respectively) or benzene, toluene, and soy FAME (2.8 mgl^{-1}, 0.8 mgl^{-1} and 54.8 mgl^{-1}, respectively), which formed both dissolved and organic phases. Abiotic controls poisoned with mercuric chloride were constructed in parallel. Biodegradation of toluene and benzene were both slower in the microcosms containing FAME than when incubated alone. Toluene was completely removed within 34 days in the presence and absence of FAME, while only 45% of benzene was removed in the presence of FAME vs 90% in the absence of FAME. The authors postulated that at least some, if not all, the benzene removal might be due to aerobic degradation since the set-up of the microcosms allowed for some oxygen leakage into the test containers. Overall, the results indicate that the presence of FAME can inhibit or at least slow the rate of biodegradation of toluene and benzene under anoxic/hypoxic conditions.

Elzarhari-Ali et al 2012 compared the rate of disappearance of components of a laboratory-constructed blend of hydrocarbons to a B20 blend of the same mixture with commercial rapeseed FAME in aerobic microcosms constructed of wet sandy soil, with and without the addition of inorganic nutrients (N and P). The addition of nutrients resulted in significantly greater hydrocarbon removal both in the hydrocarbon-only mixture and the B20 microcosms. In the microcosms without nutrients toluene was removed more rapidly from the hydrocarbon-only mixture than in the B20 microcosms, while the rate of removal of toluene was essentially the same in both with added nutrients. Although not discussed by the authors, this observation may be the result of nutrient limitation with FAME degraders outcompeting for the available nutrients.

Fuller et al 2013 reported data relating to the effect of FAME on the degradation of diesel constituents. Microcosms containing creek water were amended with diesel, FAME or a blend of FAME/diesel (B20) and aerated during incubation in a greenhouse under ambient conditions. The biodiesel was a 10:90 w/w blend of tallow and canola oil FAME. After 28 days, a higher proportion of the mass of polyaromatic hydrocarbons pyrene, fluoranthene, methylpyrenes, and C2-alkyl fluorenthenes and pyrenes were removed in the B20 blend than diesel alone. Since these PAH are not expected to significantly weather due to volatilisation, the authors concluded that this impact of biodiesel on PAH weathering might be due to photo-oxidation or aerobic degradation. However, more tests would be required to determine the mechanism.

Yassine et al 2013b conducted aerobic degradation studies on laboratory blends (B20, B40, B60 and B80) of a commercial soybean FAME and low-sulphur petroleum diesel. Microcosms were inoculated with a bacterial culture developed from a sample of activated sludge from a wastewater treatment plant mixed with two laboratory cultures capable of biodegradation of gasoline and triglycerides, which had been acclimated to FAME, diesel and biodiesel. Biodegradation rates of n-alkanes were enhanced in the FAME blends and the authors concluded that this was due to higher aqueous concentrations and bioavailability of n-alkanes in the presence of FAME. Cometabolism was discounted as a possible explanation for the increased rates on the basis that both FAME and n-alkanes are growth substrates. Further, only the specific utilisation rate was affected and not the total degradation rate and so the enhancement was not considered likely to be due to increased biomass production in the presence of FAME (this being a more favourable growth substrate than n-alkanes). The effect of FAME on aromatic compounds during these experiments was mixed. Toluene, o-xylene and tetralin, which were not degraded in the diesel and B20 microcosms during the period of incubation, were completely degraded in B40 and B80 to below the detection limit. In the B60 microcosms only ethylbenzene was degraded during the 7 day incubation in multiple replicate experiments. Mineralisation of diesel also appeared to be enhanced in the blends with FAME. The authors did not provide an explanation for the lack of expected biodegradation of toluene and other compounds in pure diesel and B20. A review of the supporting data presented in graphical format indicates that some degradation of toluene may have occurred in the pure diesel and B20 microcosms. However, the graphical data are difficult to interpret.

Borges et al 2014 conducted tests to determine whether the presence of FAME produced from soy, pork and castor oil would affect the biodegradation of benzene under anaerobic conditions. Microcosms were constructed using synthetic groundwater containing 22 mgl^{-1} sulphate and sediment (with either benzene alone or benzene plus soybean, pork or castor FAME or one of the three FAME alone). The microcosms were initially under oxidising conditions and transitioned to anaerobic conditions within one month. Only 17.7% of benzene was removed within 150 days in the benzene-only microcosms. Benzene removals in the FAME-containing microcosms were slightly less at 10.8%, 14.4% and 6.7% for soy, pork and castor FAME, respectively. Since no data from abiotic controls containing benzene were presented, benzene loss through volatilisation cannot be quantified but may be contributing to these losses. Organic acid production was also measured and was 34 to 56% less in FAME + benzene microcosms than in the FAME only microcosms. Methane production data were not presented for the benzene-containing microcosms. Since other data presented in the paper indicate that these microcosms might have been nutrient-limited, and that variability in the added sediment might contribute to observed differences, the observed effect of FAME on benzene degradation and benzene on FAME degradation cannot be assessed as significant.

Lisiecki et al 2014 conducted studies on blends of rapeseed oil FAME with petroleum diesel in sealed undisturbed saturated sand microcosms inoculated with a diesel-degrading consortium isolated from a crude oil release site. These microcosms would be expected to simulate an aquifer environment close to the top of the water table with limited oxygen transfer from the gas phase into the water phase. The microcosms were incubated for 578 days. Production of carbon dioxide was measured throughout the incubation period. At the end of the incubation, the residual fractions of aromatic hydrocarbons, aliphatic hydrocarbons, total petroleum hydrocarbons, and FAME were determined by GC-FID. A shift in the bacterial consortium was observed with one type increasing with increasing percentage of FAME in the blend and three types decreasing. In the FAME and diesel microcosms, approximately 58% and 30% of FAME and diesel were mineralised to carbon dioxide, respectively. Overall, the presence of FAME did not appear to enhance the rate or extent of diesel degradation. Rates of carbon dioxide production from the blends could generally be predicted from the FAME: diesel blend ratio and the rate of carbon dioxide production in the FAME and diesel only microcosms. The residual fractions of aromatic and aliphatic hydrocarbons were generally the same in the B10 through B80 microcosms. Approximately 35 to 40% of the initial substrate carbon was not accounted for as carbon dioxide, residual hydrocarbons or FAME, likely reflecting losses due to biomass or intermediate production, or in the case of diesel, losses through volatilisation.

Parallels may be drawn between the effect of FAME on diesel attenuation, and the effect of ethanol on benzene and BTEX plumes. Studies of BTEX plumes in the presence and absence of ethanol have shown that ethanol-containing BTEX plumes may be significantly longer than BTEX only plumes (Deeb et al 2003; Ruiz-Aquilar et al 2003; Mackay et al 2006). In part this is due to rapid biodegradation of ethanol depleting concentrations of electron acceptors (e.g. oxygen, nitrate and sulphate), thereby limiting biological attenuation to slower anaerobic processes such as fermentation. Production of high concentrations of hydrogen and acetate from fermentation can also have an inhibitory effect on benzene degradation (Rakoczy et al 2001). In a similar way, it is possible FAME may compete for electron acceptors utilised in the biodegradation of diesel, or break down to form intermediates that may inhibit the anaerobic fermentation of diesel constituents. However, to date, no data have been reported in the literature to support this theory; specific studies are needed to investigate such potential effects.

9. CONCLUSIONS & RECOMMENDATIONS

The primary objective of this review was to provide insight into the natural attenuation of FAME in the subsurface. The key conclusions are as follows:

The composition and properties of FAME in biodiesel vary according to the original FAME feedstock, the presence of additives and the blend of feedstock used in a commercial product. B100 (100% FAME) may be present at fuel production and storage sites, but the most commonly used and distributed forms of FAME-containing fuels are 5 to 20% (B5 to B20) blends of FAME with petroleum diesel.

Individual FAME compounds are of low aqueous solubility, low volatility and low mobility. FAME, based on its low bulk density and aqueous solubility, is expected to exist as a light non- aqueous phase liquid (LNAPL) in the subsurface. A FAME LNAPL source would be relatively immobile, potentially long- lived (dependent on the volume of the release), but have a relatively small region of influence. In this context, a B5 or B20 FAME/petroleum diesel blend may be expected to behave similarly to petroleum diesel in the subsurface. The mixtures of FAME that have been studied in peer review literature do not appear to enhance the solubility of hydrocarbons as a whole or individual components such as PAH or monoaromatic hydrocarbons.

Numerous laboratory studies have been conducted to investigate the biodegradation of FAME (both bulk FAME and individual compounds) from various feedstocks. These studies have utilised different test conditions, microbial inocula, amendments and analytical measurements:

- Multiple laboratory studies have demonstrated that biological mineralization of FAME to carbon dioxide occurs under aerobic conditions. Oxygen depletion in response to FAME has also been observed at the two field sites
- Multiple laboratory studies have demonstrated biodegradation of FAME under methanogenic conditions. Methane production in response to FAME has also been observed at the two field sites
- A more limited number of laboratory studies have provided evidence that biodegradation of FAME occurs through nitrate and sulphate reduction. Nitrate and sulphate depletion in response to FAME biodegradation has also been observed at one field site
- Although no laboratory studies were identified that evaluated biodegradation of FAME through iron or manganese reduction, increases in dissolved iron in response to FAME have been observed at the two field sites. No data have been reported on manganese reduction.

These observations are consistent with FAME biodegradation through all major redox processes involved in natural attenuation.

Biodegradation of FAME under anaerobic conditions has the potential to produce significant quantities of methane. In two field studies (Section 7) biodegradation of FAME was reported to be associated with high concentrations of methane in groundwater (30 mgl^{-1}) and soil gas (up to 67%). The release of significant quantities of methane into the subsurface during biodegradation could lead to a fire or explosion risk in the event that it accumulates in confined areas, such as basements or utility conduits. The potential for methane production should, therefore, be taken into account during the risk assessment of FAME release sites. Off-gassing of methane and carbon dioxide can enhance volatilization of BTEX and other volatile compounds from groundwater and thereby increase the risk of vapour intrusion into buildings. Studies conducted at other biofuel sites, such as ethanol release sites, indicate that the rate of methane formation will likely be dependent on factors such as geology, depth to water, soil moisture content and other factors. Methane production rates may vary dependent on whether the release is pure FAME, or a FAME/ petroleum diesel blend. Additional field studies are needed to determine if this is the case.

FAME is reported to increase aerobic biodegradation rates of n-alkanes in diesel at concentrations of B20 and higher but this effect, which is attributed to increased micelle formation, has not been demonstrated at field scale in the context of a subsurface release. At sites with limited electron acceptors and macronutrients (nitrogen and phosphate), microorganisms that degrade FAME have the potential to deplete available electron acceptors and nutrients resulting in an extended time for diesel biodegradation. However, the significance of this in field studies has not been reported.

While studies support the overall conclusion that FAME is readily biodegradable under both aerobic and anaerobic conditions, the specific details – rate, observation of a lag period, extent of degradation, preferential degradation of specific FAME – varied from study to study. Site–specific assessment of natural attenuation processes, in accordance with lines-of-evidence based good practice on MNA, remain necessary to demonstrate MNA on a site by site basis.

FAME has been reported to undergo relatively rapid auto-oxidation and hydrolysis in aqueous solution, with 5-10% conversion to free fatty acids and methanol over a 24 hour period. These more soluble, but equally biodegradable substances could increase the concentration of dissolved organic carbon in groundwater beyond that expected for the parent FAME. Confirmation of complete FAME biodegradation requires more than disappearance by GC because intermediates produced through auto-oxidation and hydrolysis are not detectable by standard GC methods for FAME and diesel. With the exception of methanol, these intermediates are not known to be toxic, but could continue to impact water quality. Additional work is needed to explore these effects.

Overall, natural attenuation would appear to be significant in controlling the fate, behaviour and potential risks posed by biodiesel. Significant attenuation mechanisms are likely to include sorption, autoxidation and biodegradation via a variety of redox processes: the exact role and contribution of each will depend on the nature of the release, the characteristics of the individual FAME, the FAME-diesel blend and the environmental setting. Such attenuation may have secondary impacts on the degradation of other components of the biodiesel and may in itself generate undesirable effects such as excessive methane.

To date only a limited number of studies have been published on the fate and behaviour of FAME at the field scale. Additional studies of either controlled or accidental releases of FAME or FAME-diesel mixtures would enhance understanding of the biodegradation processes discussed above and the behaviour of these processes in different geologies. Of particular interest would be additional information on the impact of FAME on the fate and transport of petroleum diesel and the production of methane.

10. GLOSSARY

Bxx	Diesel blend containing xx% v/v FAME
B100	100% v/v FAME (also called biodiesel)
BHT	Butylated Hydroxytoluene
BOD	Biological Oxygen Demand
BTEX	Benzene, Toluene, Ethylbenzene, Xylenes
CEN	European Committee for Standardisation
DOC	Dissolved Organic Carbon
EAWAG-BBD	Eigenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz - Biocatalysis/ Biodegradation Database
EN 14214	CEN European standard for FAME
EN 590	CEN European standard for regular diesel fuel
EU	European Union
FAAE	Fatty Acid Alkyl Ester
FAEE	Fatty Acid Ethyl Ester
FAME	Fatty Acid Methyl Ester
GC-FID	Gas Chromatography – Flame Ionisation Detector
GC-MS	Gas Chromatography- Mass Spectrometry
GHG	Greenhouse Gas
HC	Hydrocarbon
ITRC	Interstate Technology & Regulatory Council
LNAPL	Light Non- Aqueous Phase Liquid
MBT	Methylene bis(thiocyanate)
MGP	Manufactured Gas Plant
MNA	Monitored Natural Attenuation
NSZD	Natural Source Zone Depletion
OECD	Office of Economic Co-operation and Development
PAH	Polycyclic Aromatic Hydrocarbons
REE	Rapeseed ethyl esters
RME	Rapeseed methyl esters
SEE	Soybean ethyl esters
TBHQ	Tert-butyl hydroquinone
TEAP	Terminal Electron Accepting Process
TOC	Total Organic Carbon
UCM	Unresolved Compound Mixture

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