Conidia Bioscience



WHITE PAPER:

Biofuels, HVO and blends

To reduce environmental impact, many operators are looking for alternative fuels that can replace or blend with conventional diesel. Two of the most common options are HVO (hydrotreated vegetable oil) and B100 (FAME - Fatty Acid Methyl Ester biodiesel). But what are they and how might they be susceptible to issues such as microbial contamination.

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WHY ARE MARKETS SWITCHING TO BIOFUELS

The accelerating demand for cleaner, more sustainable energy sources has positioned biofuels as a key component of global strategies to reduce greenhouse gas emissions, enhance energy security, and support the transition away from fossil fuels.

Biofuels are renewable energy carriers derived from biomass—organic material originating from plants, animals, or waste—and can be used as direct replacements or blends with conventional petroleum fuels. Unlike fossil fuels, which release carbon that has been locked away for millions of years, biofuels can be carbon-neutral or even carbon-negative over their lifecycle, depending on the feedstock and production method. When sustainably sourced and properly managed, biofuels offer a closed carbon cycle, where the CO_2 emitted during combustion is offset by CO_2 absorbed during the growth phase of the biomass.

Technologically, biofuels are versatile. They can be blended with gasoline or diesel, used in aviation and marine transport, or even integrated into power generation systems. Many are compatible with existing infrastructure, offering a strategic advantage in accelerating decarbonization without requiring extensive retrofits.

Biofuels currently make up a relatively small portion of total energy markets (industry, buildings, transport combined), its market share is projected to double to 6% by 2030. Adoption of biofuels in the transport and aviation sectors is likely to expand rapidly over the next 5 years. In the transport sector, biofuels are expected to reach approximately 9% of energy demand by 2030. Aviation fuel will be the fastest-growing sub-sector: sustainable aviation fuel is projected to cover about 10% of aviation demand by 2030.

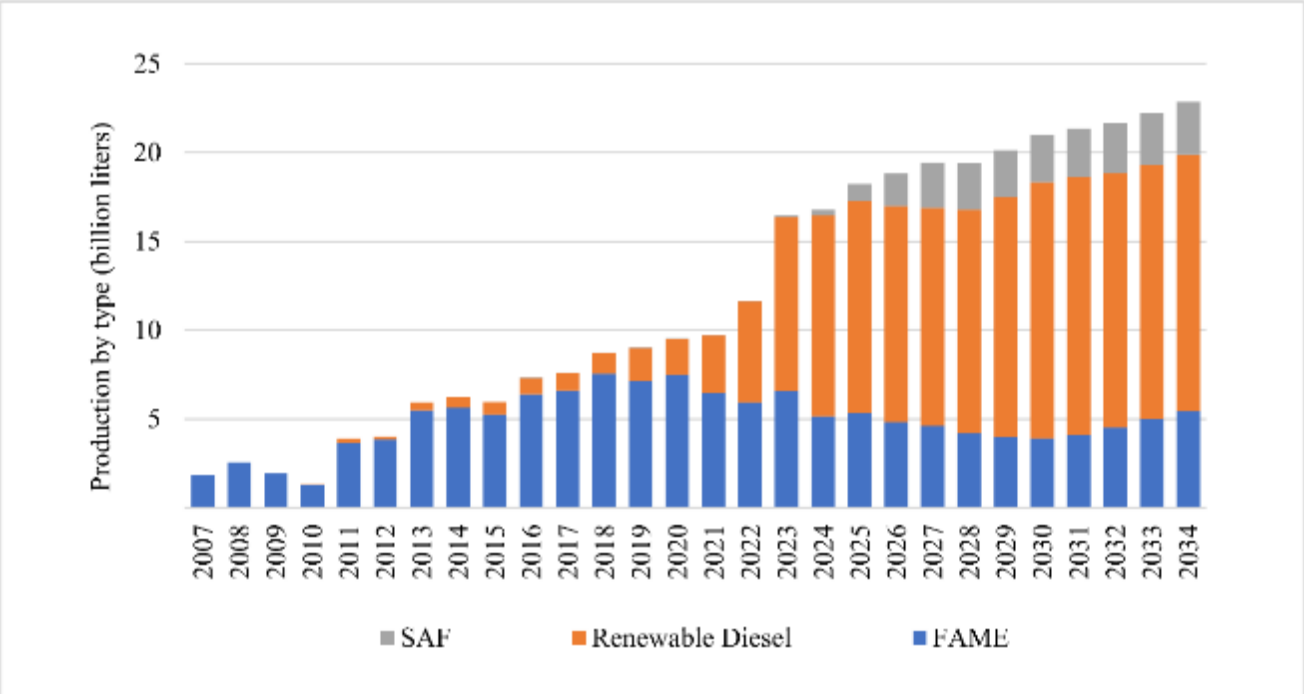
Whilst a wide range of biofuel types are commercially available, the current market is dominated by bioethanol from corn or sugarcane and biodiesel from vegetable oils. Their market share over fossil fuel is rapidly increasing as manufacturing and supply chains become more established.

The main types of biofuels currently in commercial use

Biofuel Type	Main Feedstocks	Primary Use	Market[1] Share	Commercial Status
Bioethanol	Corn, sugarcane, cellulosic biomass	Gasoline blending	60-65%	Widely used
Biodiesel (FAME)	Vegetable oils, animal fats	Diesel blending	25-30%	Widely used
Renewable Diesel (HVO)	Waste oils, fats	Drop-in diesel replacement	5-7%	Rapidly growing
Sustainable Aviation Fuel (SAF)	Waste oils, alcohols, biomass	Jet fuel blending	<1%	Emerging, strategic priority

Over the next 10 years it is expected that the production and adoption of HVO will rapidly increase. One of the key reasons for this is that it's a 'drop in' fuel and can simply be swapped with diesel without need for any modifications to engines. SAF usage is also expected to start increase as airline manufacturers start to allow higher percentages of SAF to be blended with Jet A/A-1 fuel.

Projected production rates of SAF, Renewable Diesel (HVO) and FAME over the next 10 years



Debnath, D.; Whistance, J.; Thompson, W. The U.S. Biodiesel Outlook: A Pathway Toward Sustainability. *Sustainability* **2025**, *17*, 689. https://doi.org/10.3390/su17020689

[1] Understand Energy Learning Hub Biofuels | Understand Energy Learning Hub - Stanford University Fast Facts About Biofuels · https://understand-energy.stanford.edu/energy-currencies/biofuels?

WHAT ARE THE DIFFERENT FORMS OF BIOFUELS

Biodiesel (FAME - Fatty Acid Methyl Ester)

Biodiesel is a renewable biofuel, a form of diesel fuel, derived from biological sources like vegetable oils, animal fats, or recycled greases, and consisting of long-chain fatty acid esters. Conventional biodiesel product meeting EN 14214[2] and ASTM D6571[3] product specifications have different chemistry and properties to conventional diesel fuels. Despite these, they are widely used as a blend with conventional diesel fuel or as pure product (B100).

As explained in Conidia's previous white paper[4], "Studies have shown that diesel fuels containing biodiesel content may be more susceptible to premature degradation when not properly maintained due to the chemical properties of biodiesel."

We know from the research paper "Moisture Absorption in Biodiesel and its Petro-Diesel Blends[5]" that 100% biodiesel fuel can hold 15 to 25 times more water compared to 100% diesel fuel. It also states that the biodiesel hits saturation quickly. This is because both water and biodiesel molecules are polar, so the water molecules are attracted to, and bond quickly to, the biodiesel molecules."

Biodiesel blends ranging from B7 to B100 can contain up to 2000 ppm of total water content depending on fuel temperature and atmospheric pressure. This is a cause for concern with respect to microbial growth.

Hydrotreated Vegetable Oil (HVO)

Like biodiesel, HVO is made of nonpetroleum renewable resources such as natural fats, vegetable oils (i.e. rape seed (canola) and soybean oil), and tallow greases. Much like biodiesel, HVO is derived from waste agricultural products, particularly waste vegetable oils and waste animal fats. This is why it is often referred to and qualified as "Renewable HVO Diesel" or "Green Diesel".

HVO chemistry differs from biodiesel, being composed mainly of paraffinic hydrocarbons, with no oxygen content such as FAME (Fatty Acid Methyl Esters).

Renewable HVO diesel is processed in similar way to how conventional petroleum diesel is produced, which makes them chemically similar. This means a few things:

- Because it's hydrogenated, renewable diesel doesn't contain polar compounds, and therefore users will not encounter the challenges biodiesel presents relating to low temperature performance and storage stability. HVO is less prone to oxidation and therefore is less prone to

[2] EN 14214 - Liquid petroleum products - Fatty acid methyl esters (FAME) for use in diesel engines and heating applications - Requirements and test methods
[3] ASTM D6571 - Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels
[4] WPR505 Protecting Equipment from Microbial Contamination when changing Fuel Chemistry
[5] Moisture Absorption in Biodiesel and its Petro-Diesel Blends - Published by the American Society of Agricultural and Biological Engineers, St. Joseph, Michigan www.asabe.org

formation of undesirable compounds such as hydroperoxides, carboxylic acids etc. compared to biodiesel

- Renewable diesel also burns cleaner than biodiesel
- Renewable diesel can be used in engines that are designed to run on conventional diesel fuel with no blending required or change to the existing infrastructure
- Renewable diesel has similar interaction with water than regular diesel fuel

As a drop-in alternative to regular diesel, almost any engine, piece of equipment or vehicle that can run on “white” or “red” diesel, can run on HVO. It has been approved by many engine and vehicle OEMs. Unlike pure biodiesel (100% Biodiesel), it can be used without any engine modifications, either neat (100 % HVO) or blended with regular diesel at any ratio, providing that the density and aromatic compounds and concentration is adequate.

Renewable diesel is purely a synthetic fuel and as such, it is stable and suitable to long storage conditions (up to 10 years). However, like traditional hydrocarbon derived fuels, renewable diesel will absorb a certain amount of moisture with a typical total water content of 200 mg/kg (ppm) at time of production. A Clear & Bright HVO can contain as low as 40 mg/kg (40 ppm) total water content.

When exposed to excess moisture and water, HVO material will behave like any other fuel where, upon saturation, water will deposit in the bottom of tanks to form a second layer (water layer) providing the needed oxygen and nutrients for microorganisms to proliferate.

Diesel and HVO fuel storage tanks must be managed to minimize the presence of moisture and free water. Free water should not be able to accumulate in tanks bottom for any extended period.

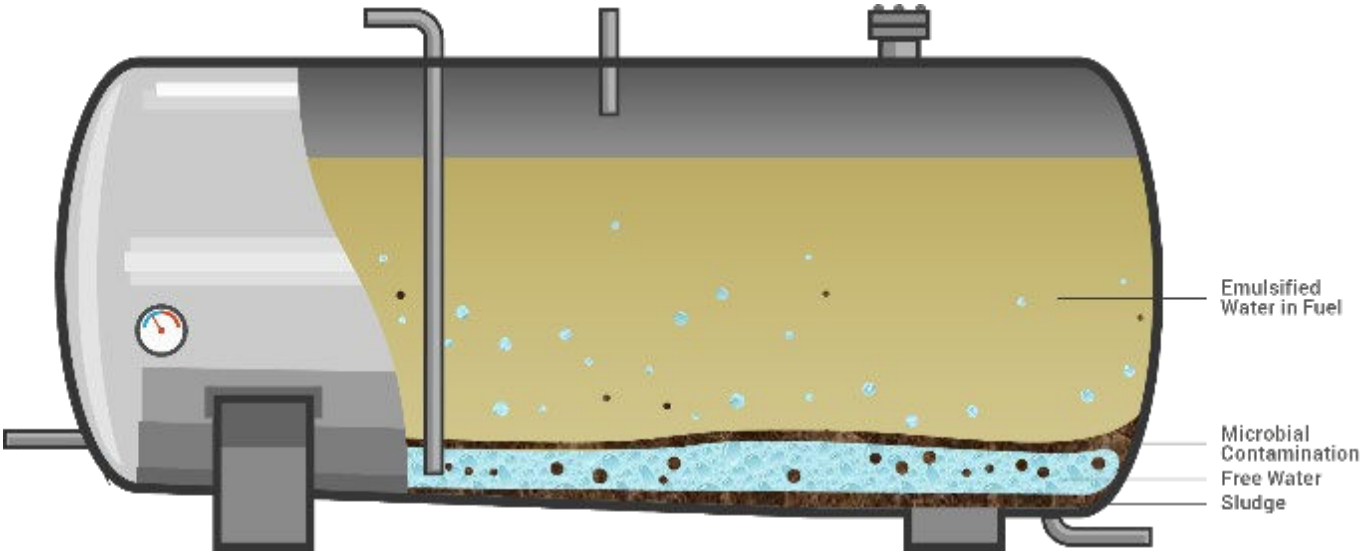
Bioethanol & Sustainable Aviation Fuel (SAF)

Note: For the purposes of this paper, we will not be investigating Bioethanol & SAF as we are here focused primarily on alternatives to Diesel & Marine grade fuels. Further investigation on these fuels will take place in the future.

BIOFUELS, WATER AND MICROBIAL CONTAMINATION

Whilst microbes are inherent in most storage and distribution systems, proliferation of microbes requires certain conditions. Three elements are typically necessary to initiate and sustain microbial growth in any fuel system:

- **fuel** – used for energy
- **microbes** – that are ubiquitous in the surrounding environment
- **water** – either free or emulsified (water finely dispersed in the fuel layer)



Ways Microbes can enter a fuel system

1

During Production

Contamination may originate in production processes, e.g. non-sterile process water

2

Transport & Storage

Introduction of air, temperature fluctuations, or residual water in tanks provides a favourable growth environment

3

End-Use Equipment

Low turnover rates and poor fuel management practices contribute to microbial colonization

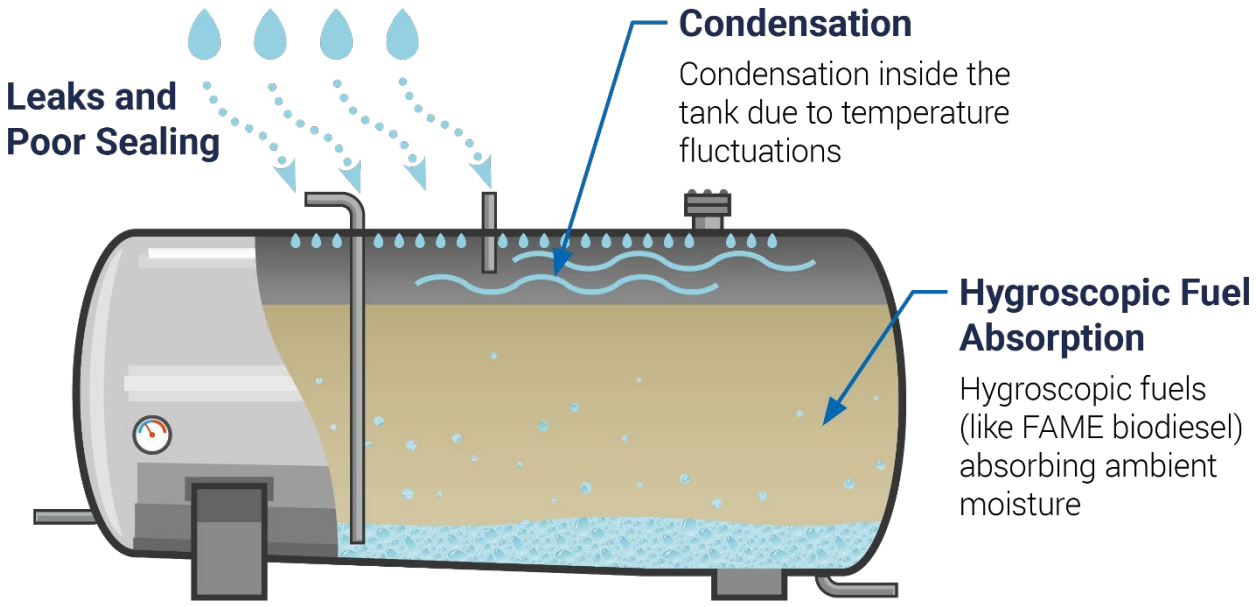
Water

Dormant microbes (spores) which are almost always present in fuel systems come to 'life' and start growing in the presence of water and correct environmental conditions.

Water can enter fuel storage tanks through several pathways, each of which poses a risk for microbial growth and fuel degradation:

- **Condensation:** Temperature fluctuations cause moisture in the air to condense on the inner surfaces of tanks, especially in partially filled systems.
- **Leaks and Poor Sealing:** Faulty gaskets, corroded fittings, or damaged tank components allow rainwater or groundwater to infiltrate the tank.
- **Hygroscopic Fuel Absorption:** Fuels such as biodiesel and ethanol are hygroscopic and can absorb moisture directly from ambient air during handling or storage.

Water Ingress Mechanisms in Fuel Tanks



Water can be present in fuel systems in several forms such as a water layer at the tank bottom or water-in-fuel emulsion. Both forms of water will lead to growth and proliferation of microorganisms. Water solubility is fuel temperature dependent, therefore greater concentration of emulsified water will be encountered at higher ambient temperatures during fuel storage. Alternatively, fuel stored in colder environment, e.g. below 0°C will be less prone to microorganism proliferation.

The presence of bacteria, yeasts, and fungi in biofuels can result in fuel degradation, corrosion of storage systems, filter blockages, and even engine failure.

Why biodiesels are particularly susceptible to microbial contamination?

As biodiesels gain momentum as a sustainable alternative to fossil fuels, one issue that is often underestimated is the increased risk of microbial contamination. Biofuels—especially biodiesel and ethanol—are more prone to microbial contamination than conventional fossil fuels.

Key factors that lead to higher susceptibility of biofuels to microbial contamination compared to fossil fuels

Factor	Biofuels	Fossil Fuels
Water absorption	High	Low
Nutrient availability	Rich in oxygenated organics	Poor
Microbial growth support	High	Low
Storage risk	Higher	Lower
Stability (oxidation)	Lower	Higher

Higher Water Affinity (Hygroscopicity)

Biofuels—particularly biodiesel (FAME) and ethanol—are hygroscopic, meaning they readily absorb moisture from ambient air. The presence of water is the single most critical factor enabling microbial activity in fuel systems. Once water accumulates (through condensation, absorption, or ingress), it creates an aqueous layer at the tank bottom or a water-in-fuel emulsion, both of which support microbial colonization and biofilm formation.

In contrast, fossil fuels affinity for water absorption is significantly less than biofuels, thereby reducing microbial viability.

Biodegradable Carbon Sources

Biofuels contain oxygenated organic compounds, such as fatty acid methyl esters in FAME and simple alcohols in ethanol. These compounds are easily metabolized by bacteria, yeasts, and fungi, making biofuels an ideal nutrient source. Fossil fuels, by comparison, are more chemically stable and contain longer-chain hydrocarbons that are less accessible or biodegradable by most microbes.

Oxidative Instability

Biodiesel is inherently less stable than petroleum diesel. It degrades more rapidly in the presence of oxygen, forming acids, peroxides, and polymers that not only compromise fuel quality but also provide additional substrates for microbial metabolism. Fossil fuels, especially refined diesel and jet fuel, degrade more slowly and are less supportive of microbial ecosystems.

Emulsification and Phase Separation

Biofuels are more prone to emulsion formation, reducing the interfacial tension of fuel. By lowering the interfacial tension of two dissimilar liquids, this phenomena facilitates the formation of free water. Once the emulsion is saturated, a phase separation will occur, generating a free water phase, creating an environment where microbes can thrive. The greater amount of FAME concentration in biodiesel blends will increase this emulsification process. In addition, there is a negative effect of emulsified fuel on filter water coalescer technology rending it inefficient, this is why the maritime industry uses centrifugal systems to help in separating and eliminating water from systems.

Storage and Handling Conditions

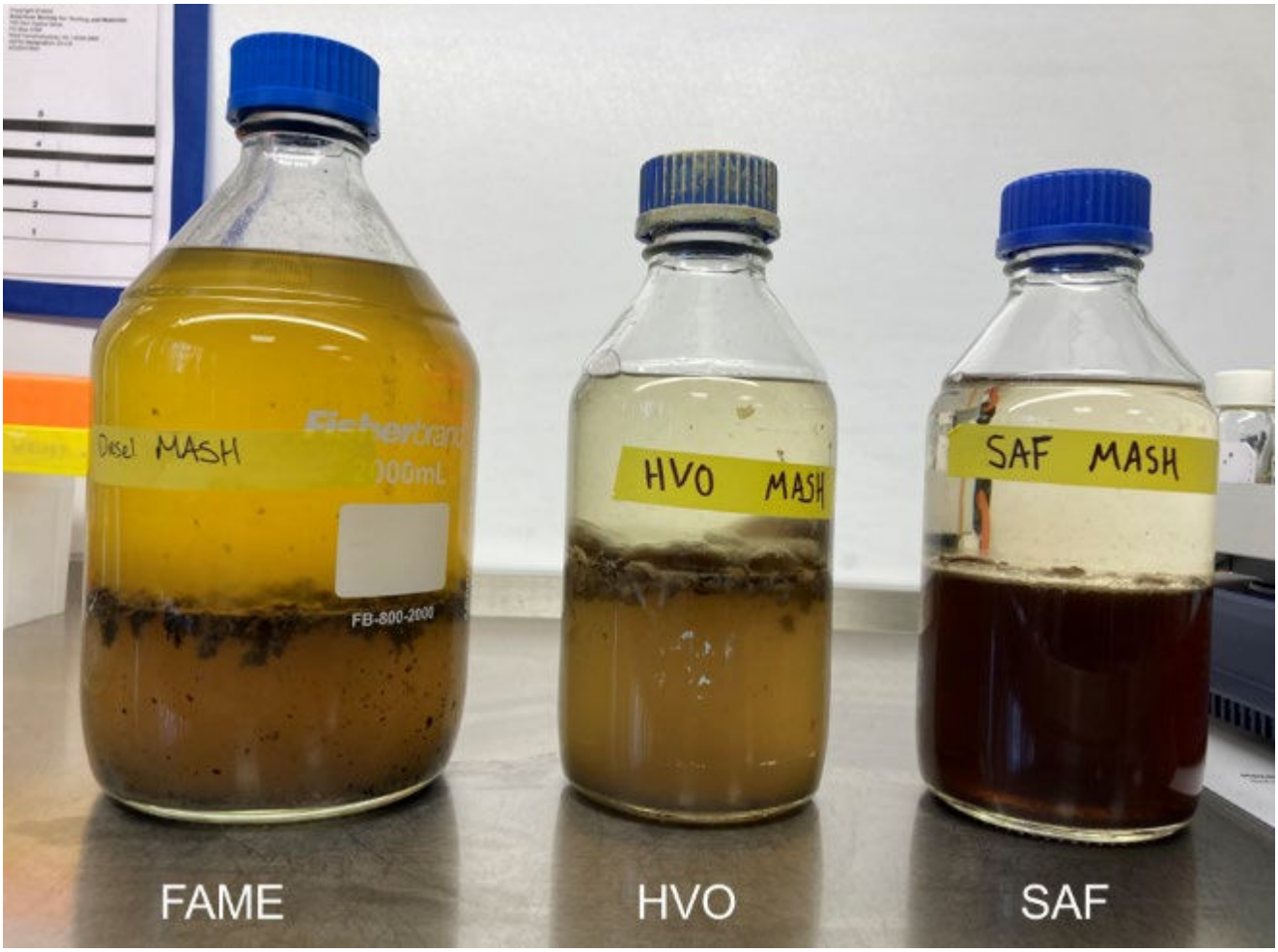
Biofuels are often stored in decentralized or low-turnover environments (e.g., agricultural settings, fleet depots), where temperature fluctuations, long storage times, and infrequent maintenance contribute to microbial growth. Fossil fuels, due to higher turnover and centralized distribution, are often less exposed to such conditions.

These conditions favour microbial growth more than the typically high-turnover environments of fossil fuel storage. This is why we do not recommend to store biodiesel blends for more than 6 months before use. Microbial growth in biofuel systems is not only a quality control issue but a multifaceted operational concern.

LABORATORY TESTING OF BIOFUELS

FAME, HVO and SAF were evaluated for susceptibility of microbial growth by simulating conditions that would be found in a typical fuel tank. A blend of the 7 commonly found organisms in fuel systems[6] was added to a mixture of fuel and an aqueous phase (microbial culture medium). This mixture is then left at Room Temperature for several weeks and visually inspected to assess the level of microbial growth.

Mature microbial growth in FAME, HVO and SAF fuels



[6] *Burkholderia* sp. , *Yarrowia lipolytica*, *Hormoconis resinae* (*Amorphoteca resinae*), *Bacillus pulmilus*, *Pseudomonas aeruginosa*, *Candida tropicalis*, *Methylobacterium* sp.

Test Results

Susceptibility of biofuels to microbial growth

Once sufficient growth has been established, fuel and water phase samples are tested to determine levels of contamination. Samples are 'plated' on solid microbial culture media to determine cfu counts.



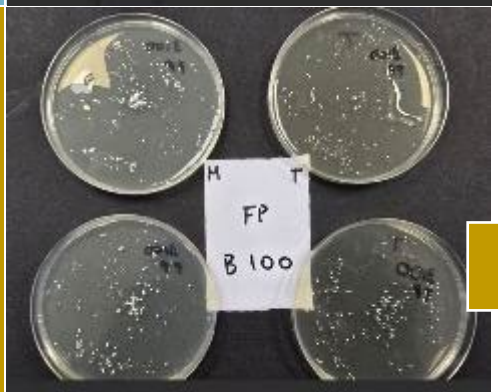
B100 Diesel (FAME)

Negative Control
Clean fuel with no contamination



Contaminated B100 Diesel

Fuel phase sample

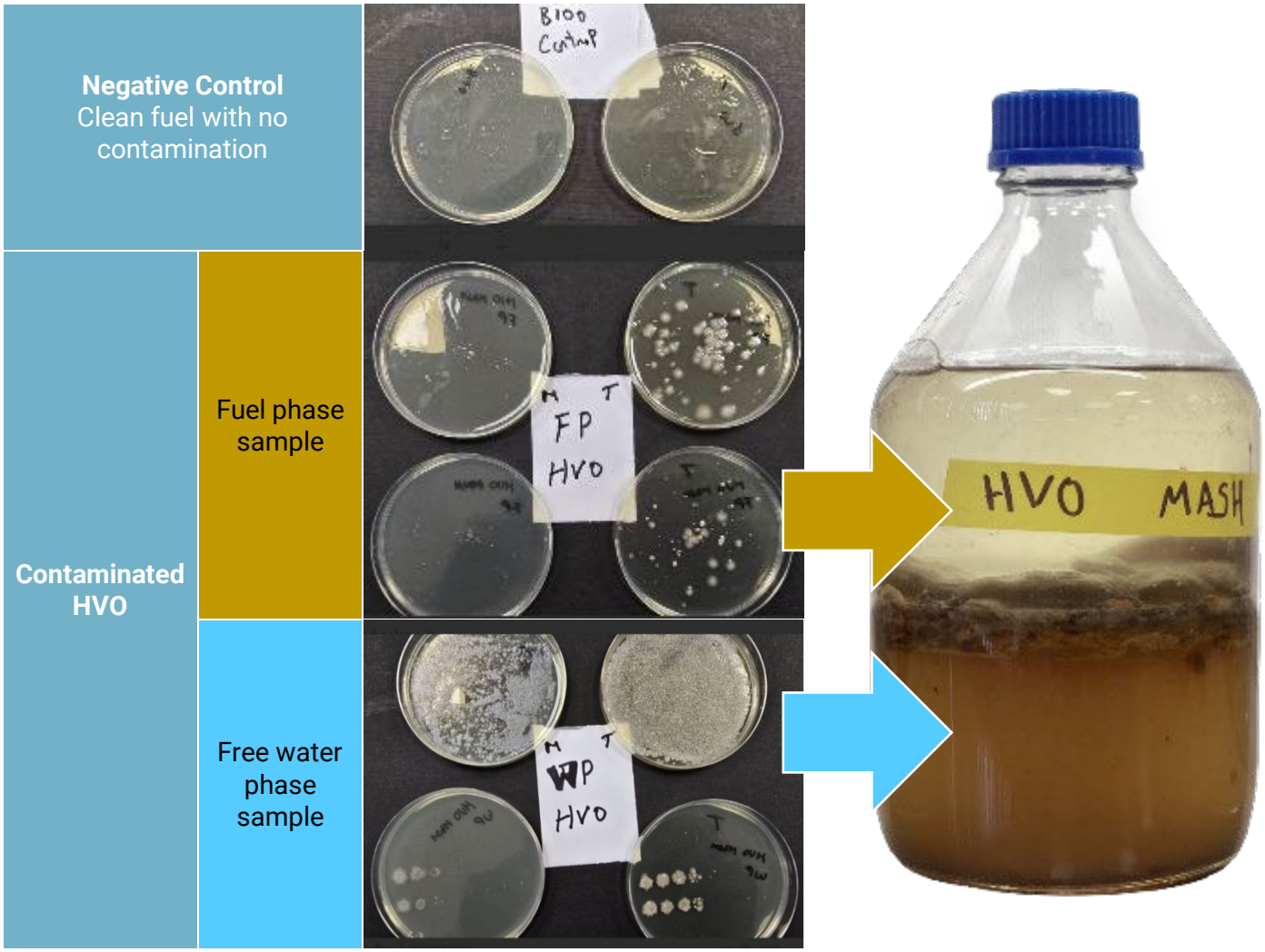


Free water phase sample





Hydrotreated Vegetable Oil (HVO)



These results demonstrate that all biofuels are susceptible to microbial contamination. In particular debunking the myth that HVO somehow would not suffer from microbial contamination issues.

Compatibility of FUELSTAT® *One* with Biofuels



Contaminated sample of HVO and 100% FAME were prepared in the laboratory. Contamination levels of both the fuel and water phases was confirmed by culturing the samples on agar plates.

The samples were then tested with FUELSTAT® *One* using contaminated samples of both B100 (FAME) and HVO in fuel and water phases.

FUELSTAT® *One* test results

Fuel Type	Sample Type	CFU/ml (modified IP385)	FUELSTAT One
Negative Control	Negative Control	0	Negligible Contamination
Contaminated B100 Diesel	Fuel phase sample	9.1E+03	Heavy Contamination
	Free water phase sample	2.9E+06	Heavy Contamination
Contaminated HVO	Fuel phase sample	3.4E+03	Heavy Contamination
	Free water phase sample	6.0E+04	Heavy Contamination

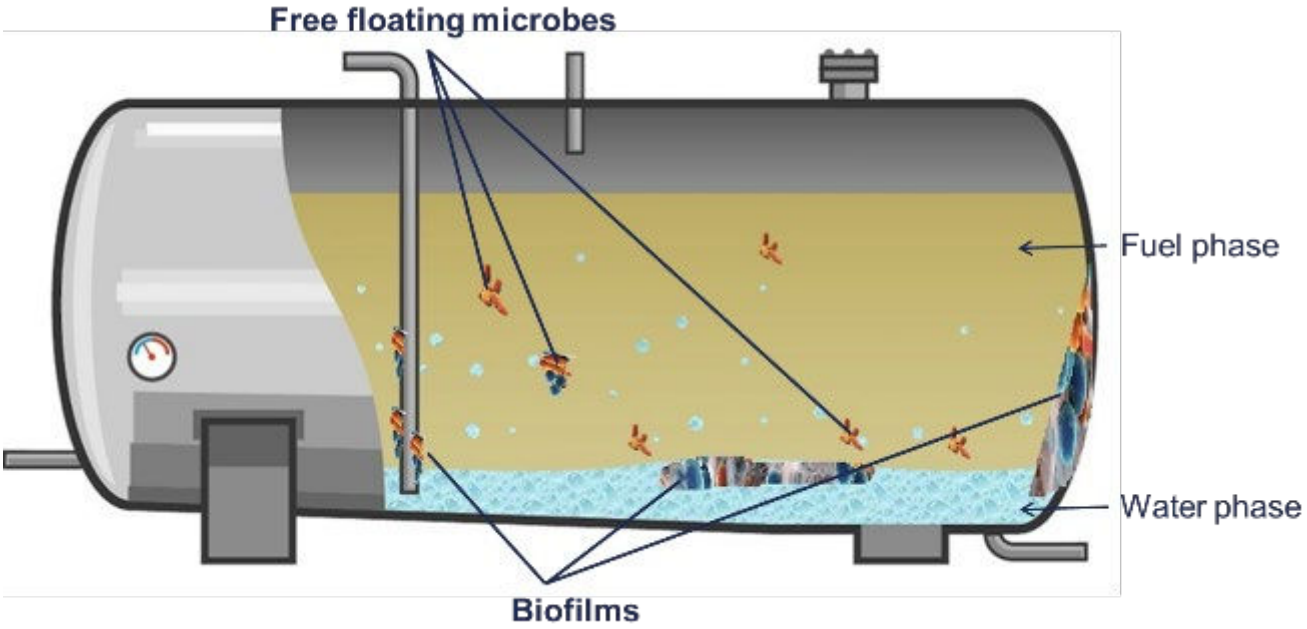
Clearly FUELSTAT® *One* performs equally well in Biofuels as well as fossil Diesel fuels indicating that this is a universal solution for fuel testing, whatever blends are being stored.

FACTORS THAT MAKE FUELSTAT® *One* A PREFERRED TESTING SOLUTION

Biofilms

Microbial biofilms consist of microbial cells embedded in a gel like matrix (extracellular polymeric material). EPS can be composed of polysaccharides, proteins, lipids, and extracellular DNA created by the microbes.

Microbes form biofilms as a survival strategy in response to environmental conditions. They provide microbes with protection, stability, and a better chance of thriving in harsh or competitive environments. Fuel is a harsh environment for microbes to exist in and hence once microbes start growing, they rapidly start establishing biofilms. In fuel biofilms exist in two forms; floating biofilms that are typically found at fuel water interface and attached biofilms.

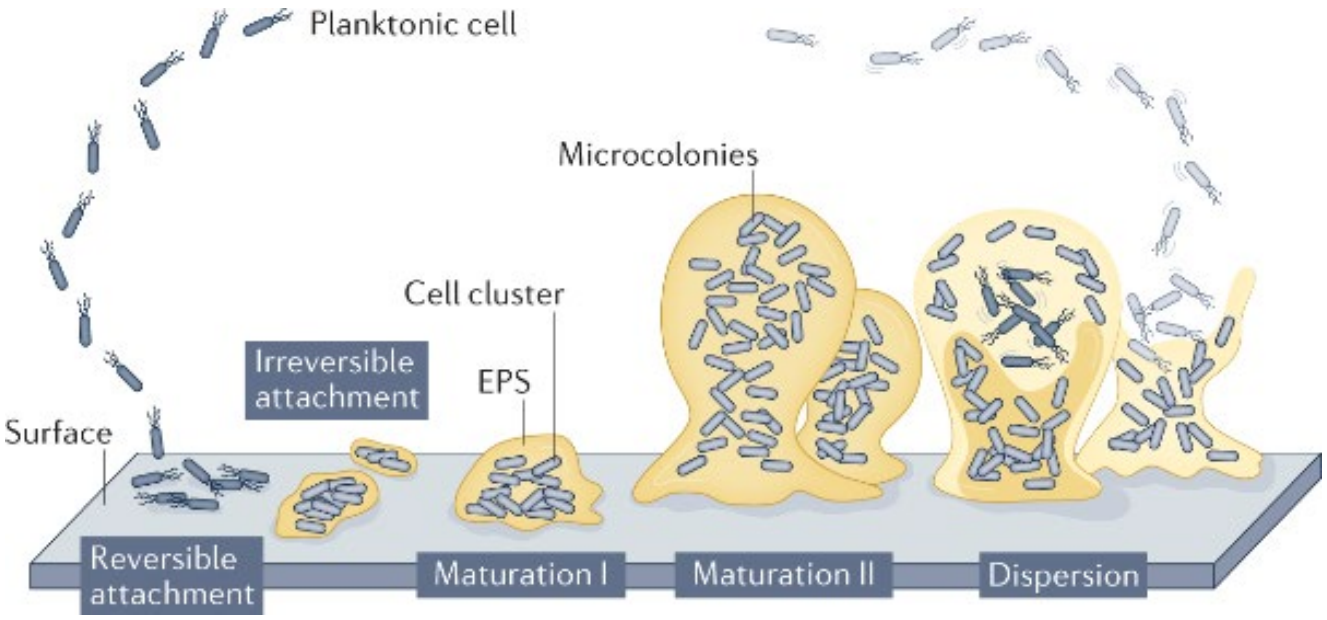


In a contaminated fuel tank microbial growth will be present in the form of biofilms (at the fuel water interface and attached to surfaces) and free-floating cells. The majority of microorganisms will be present in the water phase and at the fuel water interface as these areas are nutrient rich.

Life cycle of biofilms

Biofilm formation can be broken down into 3 key stages:

1. Attachment: Biofilm development begins with the adhesion of planktonic (free-floating) cells to a surface, followed by irreversible attachment and matrix secretion.
2. Growth and Maturation: Over time, biofilms evolve into complex, three-dimensional structures with channels that facilitate nutrient and waste transport.
3. Dispersion: Mature biofilms periodically release cells into the surrounding environment, enabling colonization of new surfaces.



Sauer, K., Stoodley, P., Goeres, D.M. *et al.* The biofilm life cycle: expanding the conceptual model of biofilm formation. *Nat Rev Microbiol* **20**, 608–620 (2022). https://doi.org/10.1038/s41579-022-00767-0

Biofilm formation in fuel tanks is a progressive microbial process that begins when bacteria and fungi colonise water-fuel interfaces, typically where water accumulates due to condensation, leakage, or hygroscopic fuel absorption. Microorganisms adhere to tank surfaces and secrete extracellular polymeric substances (EPS), creating a sticky matrix that anchors them in place and facilitates further microbial attachment.

Impact of biofilms on fuel systems

Biofilms can cause a wide range of issues in fuel systems including:

- Filter plugging
- Pump failures
- Plugging of small orifices and lines
- Sluggish response of servo-valves and equipment having small orifices
- Nozzle fouling
- Engine stalling
- Sealant and elastomeric materials being deteriorated
- Epoxy material being deteriorated
- Corrosion of tank floors, sidewalls, and tubing
- Fiberglass material being deteriorated

Biofouling of Fuel in Storage Tank



In addition to these physical impact's biofilms can lead to negative changes in fuel properties such as:

- Appearance, i.e., not being Clear & Bright
- Acidity number (pH <4)
- Oxidative stability
- Viscosity and viscosity index,
- Water separability

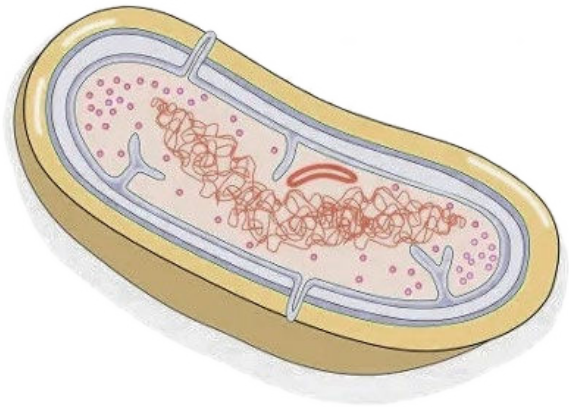


Fuel Tank Corrosion

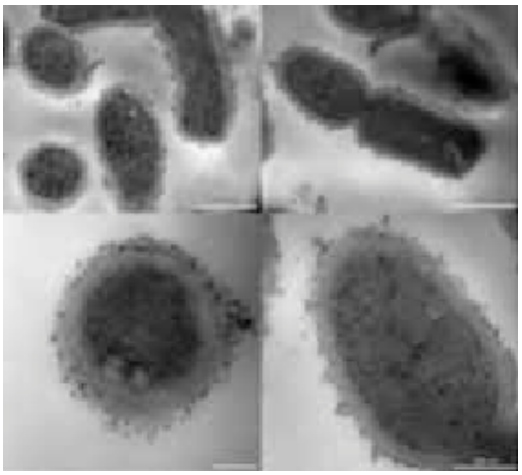
Once established biofilms confer protection against biocides, antibiotics, desiccation, and shear stress, making microbial populations within them up to 1,000 times[7] more resistant than their planktonic counterparts. This makes them very hard to remove. Biocides on their own are unable to penetrate the thick mucus EPS layer and only temporarily halt the growth of microbes. To effectively remove biofilms a combination of physical and chemical treatments is required e.g. scrubbing and biocide treatment.

Why FUELSTAT® *One* produces consistent test results across such a wide range of fuel types

Carbohydrates as a highly sensitive and broad range biomarker for microorganisms and biofilms.



Cross-section of a Microbial cell structure
Yellow outer layer indicates Polysaccharide (Carbohydrate)



SEM of Microbes showing the
paler Carbohydrate outer layer

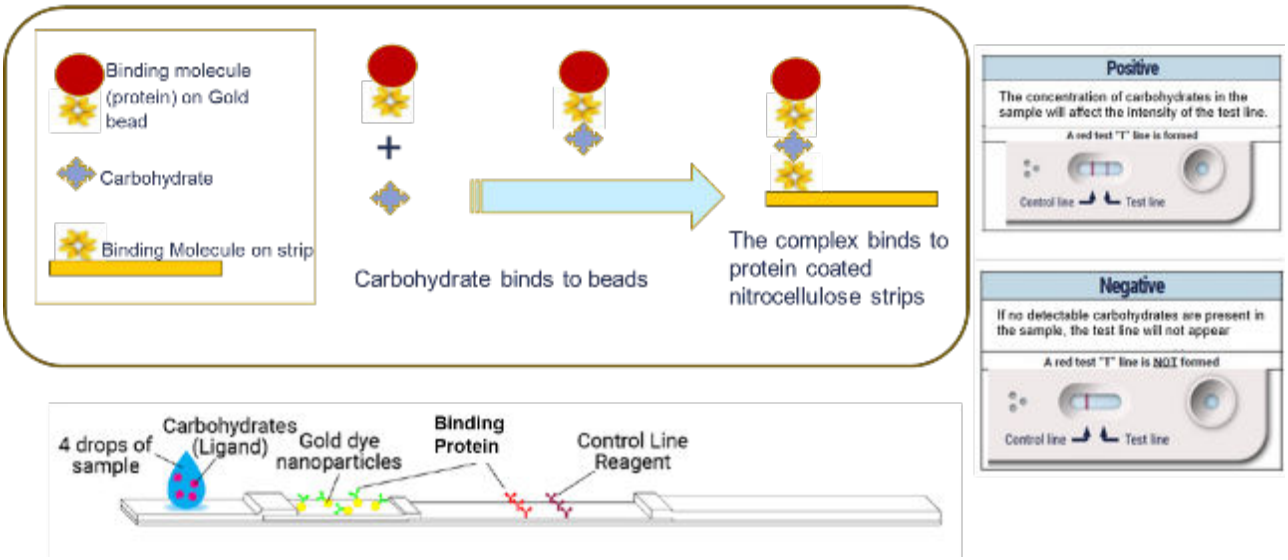
[7] Ref: Ballén, V.; Cepas, V.; Ratia, C.; Gabasa, Y.; Soto, S.M. Clinical *Escherichia coli:* From Biofilm Formation to New Antibiofilm Strategies. *Microorganisms* **2022**, *10*, 1103. https://doi.org/10.3390/microorganisms10061103

FUELSTAT® *One* detects complex carbohydrates (polysaccharides) that are produced by microorganisms (bacteria, fungi, and yeast). Polysaccharides play diverse roles, from energy storage within cells to structural components of cell walls and extracellular secretions. Microbial polysaccharides are a key component of biofilms and can represent 50-90% of the total biofilm mass[8]. The abundance of polysaccharides in and around microbes make it a highly effective and sensitive biomarker for the presence of microbial growth and associated biofilms.

FUELSTAT® *One* detects carbohydrates in the fuel sample, indicating the presence of microbial contamination, but how does the test work? Conidia's new test is a lectin-based test that uses mannan binding lectin (MBL) to detect polysaccharides (complex carbohydrates) within the samples. MBL is a protein that plays a crucial role in the innate immune system. It acts as a pattern recognition molecule, binding to carbohydrates on the surface of pathogens like bacteria, viruses, and fungi.

Lateral Flow Test technology

The FUELSTAT® *One* lateral flow test (LFT) is configured in the same way as an antibody-based sandwich test. Gold nanoparticles are labelled with MBL. If the sample contains polysaccharides, those will bind to the MBL and run along a cellulose strip. Then the polysaccharide will bind to the captured MBL (immobilised on the on the test line) producing a visible signal. A crucial component of the test is the aqueous test buffer solution. The buffer is specifically designed to efficiently extract polysaccharides present in a fuel sample. The test can then be quantified using a mobile phone app or a dedicated reader. The test can be performed and read onsite within 20-30 minutes without the need for any specialist equipment.



If carbohydrates are present in the fuel, they will bind to the protein attached to the gold and travel on the strip until they are caught again by the protein fixed on the strip. Creating a line that we can see. We will then use the intensity of these lines to give a result.

[8] Ref: Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. Microorganisms. 2023 Jun 19;11(6):1614. doi: 10.3390/microorganisms11061614.

REDUCING THE RISK OF PROBLEMS ASSOCIATED WITH MICROBIAL CONTAMINATION

As previously discussed, in order for microbes to proliferate they require water to be present in the fuel system. Fuel being supplied should be checked to ensure it does not contain water or contaminants and tanks maintained to ensure minimal water ingress. Regular water draining is recommended where the tank design allows.

Water removal

Preventing the oversaturation of fuel water content can be accomplished by using Aquafighter® water removal filters, see https://conidia.com/aquafighter/. Aquafighter® filters are designed to absorb emulsified water (water finely dispersed in the fuel layer) so the water is not available for microbial growth.



Photo 6 – Aquafighter® removing emulsified water

Testing

Regular testing for microbial contamination using FUELSTAT® test kits is also recommended, see https://conidia.com/fuelstat/fuelstat-one. FUELSTAT® is a sensitive test that can assess the presence of fuel microorganisms beyond acceptable limits within minutes. The test will provide you with an indication of level of contamination, if present.



Photo 7 – FUELSTAT® *One* test kit

Regular monitoring and testing of your fuelling system by keeping your fuel contaminant-free will:

- Increase the operational life service of equipment
- Minimize costly downtime of equipment
- Increase revenues
- Optimize engine performance engine
- Minimize corrosion in fuel tanks and fuel tank systems

Who we are:

Conidia Bioscience research, design, develop, manufacture and globally distribute innovative solutions for maintenance of fuel systems. Our products are designed to offer practical and easy to use, industry leading solutions to both maintain and monitor the stored fuel in your system, whether that be a fixed ground installed diesel fuel tank, or in an aircraft wing. Through simple steps of ensuring water doesn't develop within stored fuel, and instant testing at the tank, we keep fuel contamination free and ready to use, avoiding costly remedial action and fuel system failures.

FUELSTAT® fuel tests are developed, manufactured and marketed by Conidia Bioscience Limited. With headquarters in the UK and subsidiary in the USA, Conidia Bioscience was founded in early 2000's by experts in immunoassay techniques and holds the internationally patented intellectual property for FUELSTAT®.

Conidia Bioscience manages the global distribution of Aquafighter® water removal filters for Diesel and middle distillate fuels.

Where to find us

FUELSTAT® and Aquafighter® are distributed globally by a network of specialist distributors covering the major sectors. Contact info@conidia.com who will arrange for a distributor to support you.

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