New Horizons on Fuel Biodeterioration Mitigation Technologies

WWEC

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Biodegradation

"The process by which living organisms convert complex organic compounds into simpler substances aerobically or anaerobically"

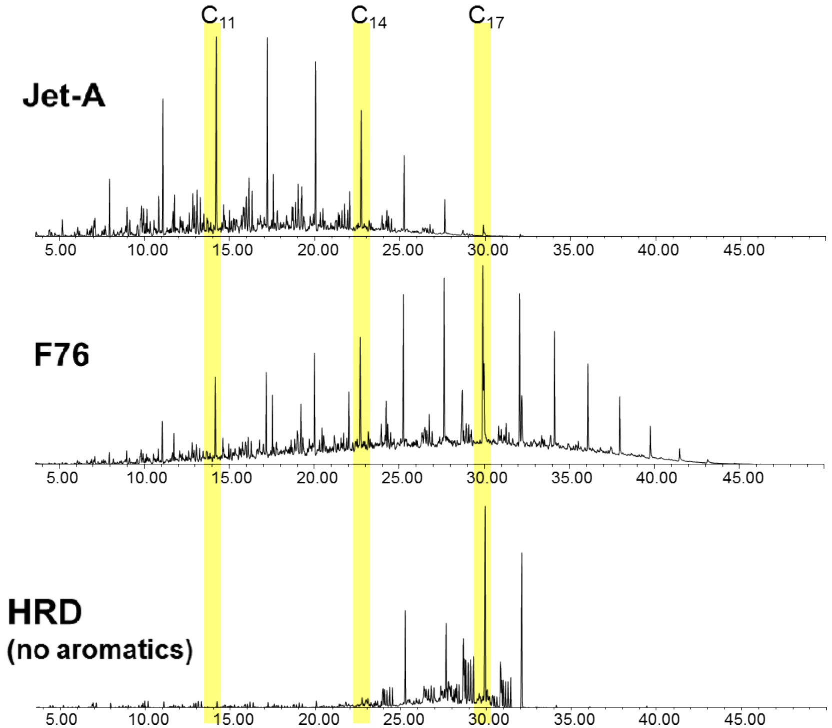
The Ugly:

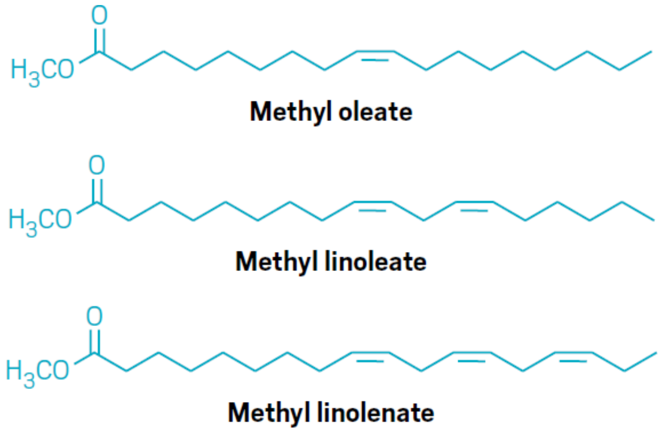
- Biocontamination (Biofouling)
  - Colonization and proliferation
- Biodeterioration
  - Physical and chemical manifestations
    - Fuel emulsification
    - Filter clogging
    - Corrosion
    - Degraded fuel properties
    - Tank contamination
    - Degraded materials & Coatings
    - Probe fouling
    - Potential vehicular issues

Role of Fuel Composition in Biodeterioration

Factors

- Hydrocarbon composition
- Fatty acid methyl esters (FAME, a.k.a. Biodiesel)
- Water content
- Dissolved oxygen
- Additives (e.g., DiEGME)
- Trace compounds and contaminants
    - Nutrient availability



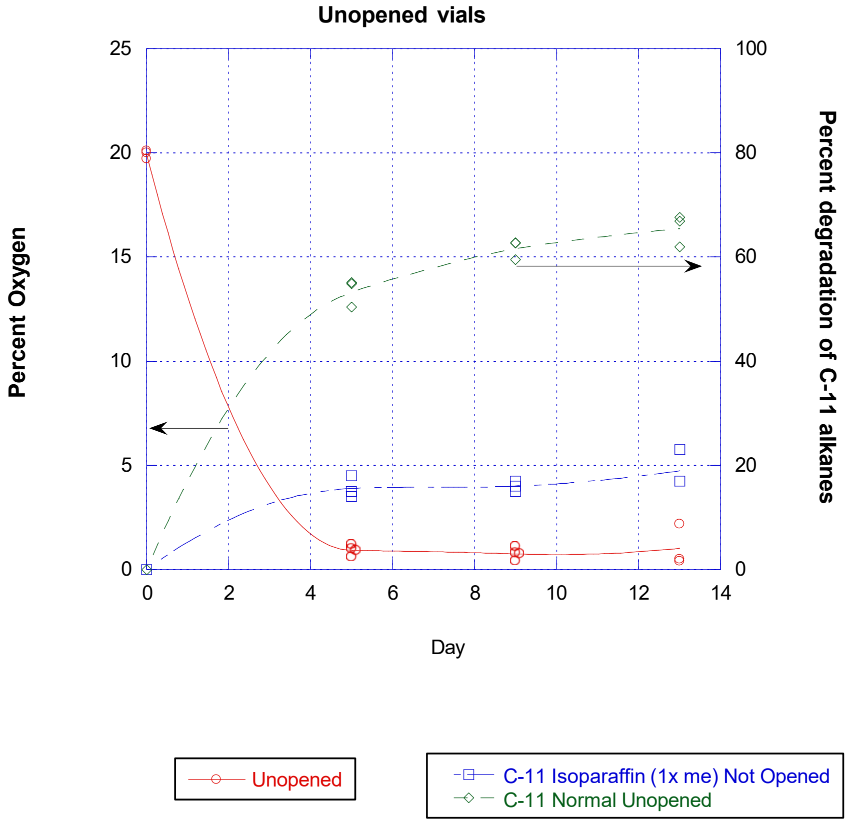


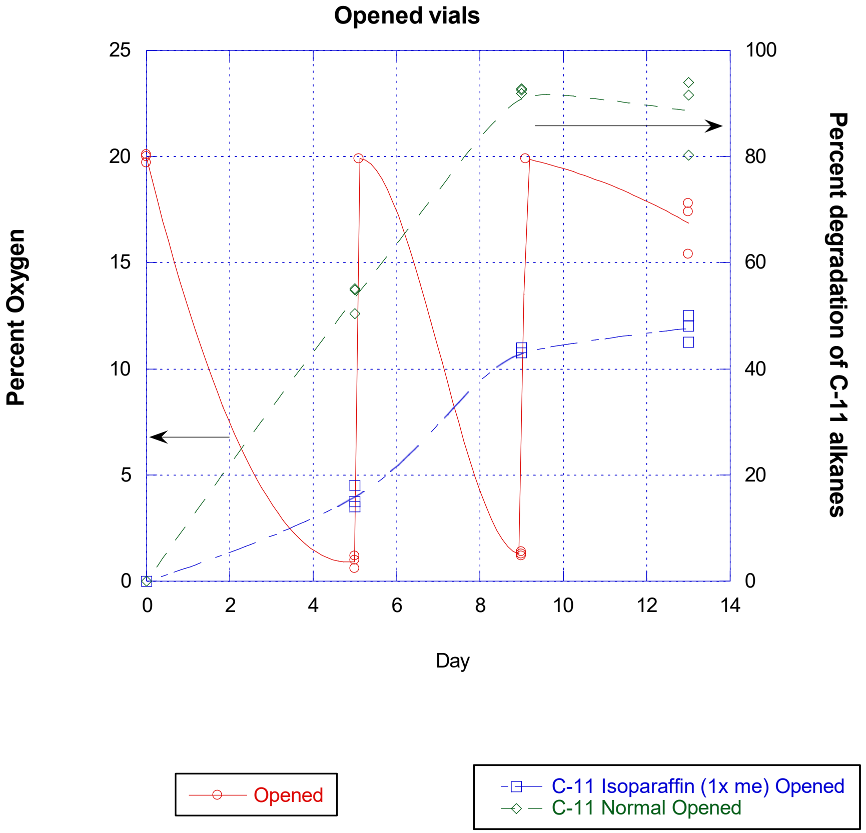
These fatty acid methyl esters, which are common in biodiesel, are readily metabolized by some microbes and enable the organisms to grow in fuel.

MARCH 5, 2018 | CEN.ACS.ORG

Effect of Oxygen Deprivation on Hydrocarbon Degradation

Yarrowia lipolytica

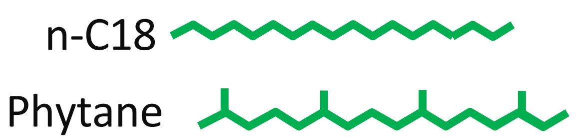


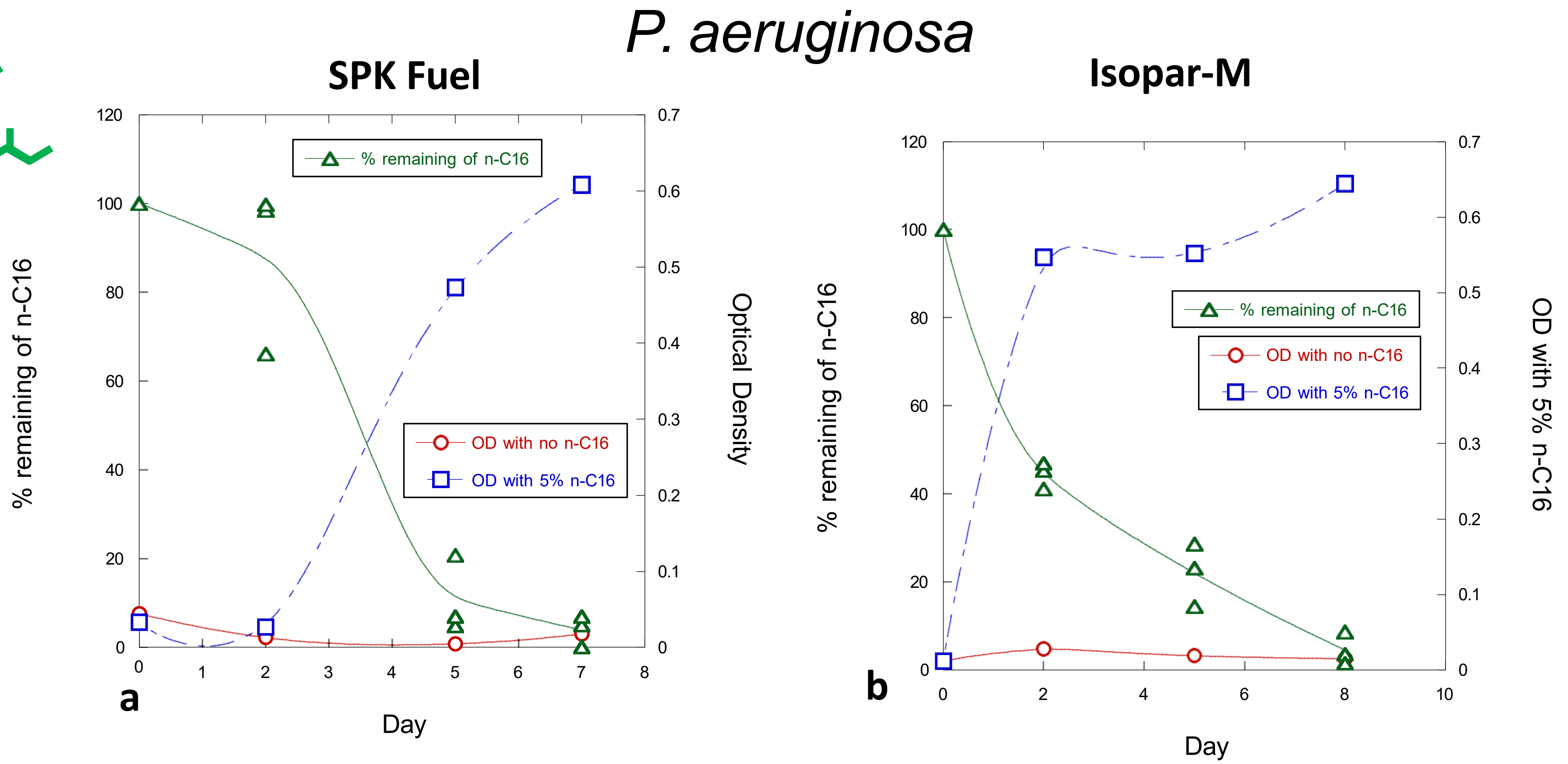


Striebich et al. 2014. Characterization of the F-76 diesel and Jet-A aviation fuel hydrocarbon degradation profiles of Pseudomonas aeruginosa and Marinobacter hydrocarbonoclasticus. *International Biodeterioration & Biodegradation*, *93*, pp.33-43.

Isoparaffinic Fuels are Recalcitrant to Biodegradation

Bio-Derived Does Not Equal to More Biodegradable



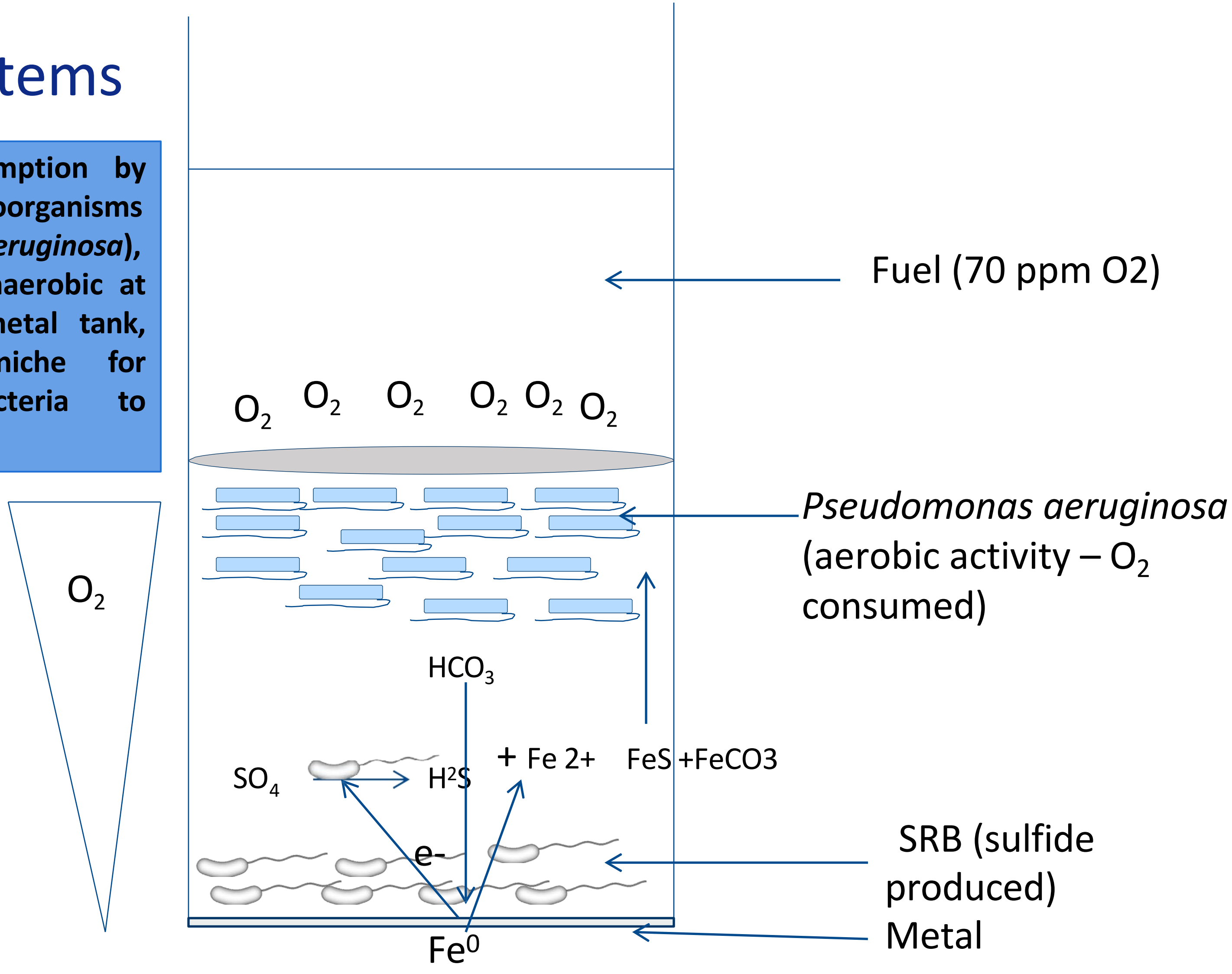


Striebich et al. 2014. Characterization of the F-76 diesel and Jet-A aviation fuel hydrocarbon degradation profiles of Pseudomonas aeruginosa and Marinobacter hydrocarbonoclasticus. *International Biodeterioration & Biodegradation*, *93*, pp.33-43.

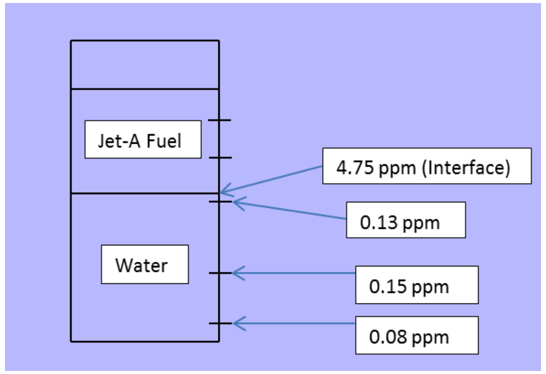
Aerobic and Anaerobic Conditions in the Fuel Tank

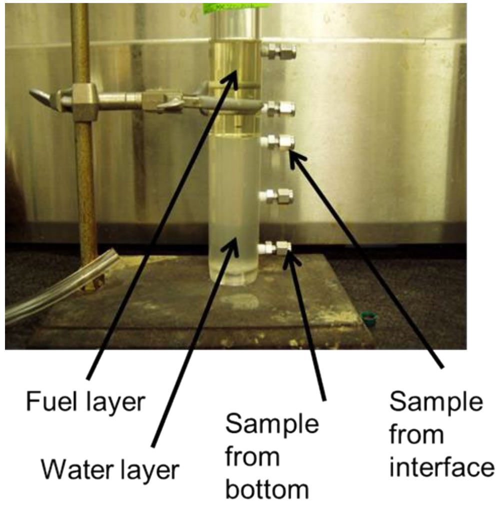
Tractable Systems

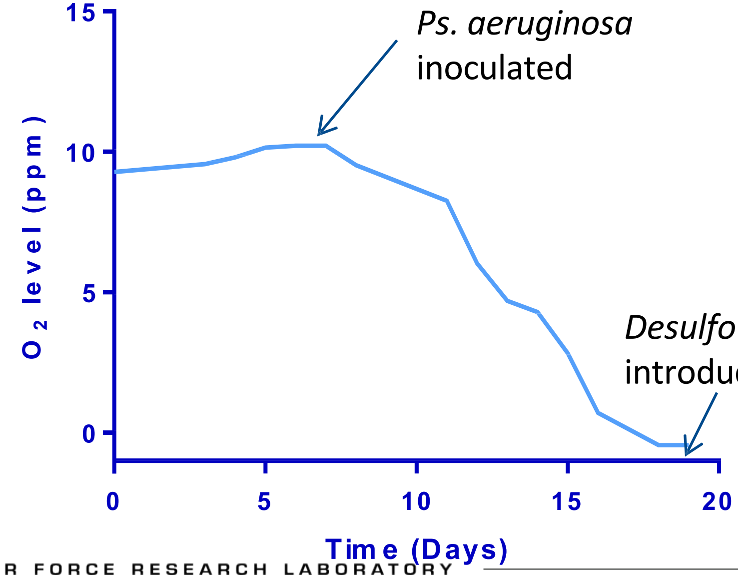
Due to oxygen consumption by aerobic microorganisms (*Pseudomonas aeruginosa*), biofilms are largely anaerobic at the bottom of the metal tank, which creates a niche for anaerobic SRB bacteria to proliferate.



Depletion of Oxygen by *P. aeruginosa* Allows SRB Growth



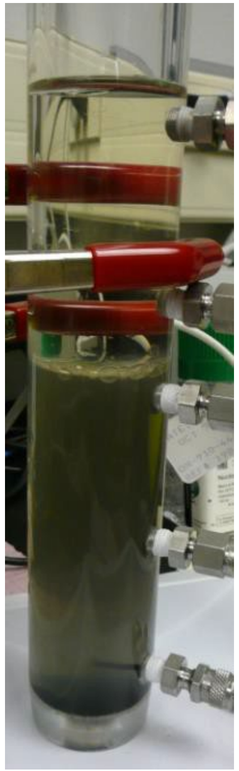




P. aeruginosa & Desulfovibrio alaskensis

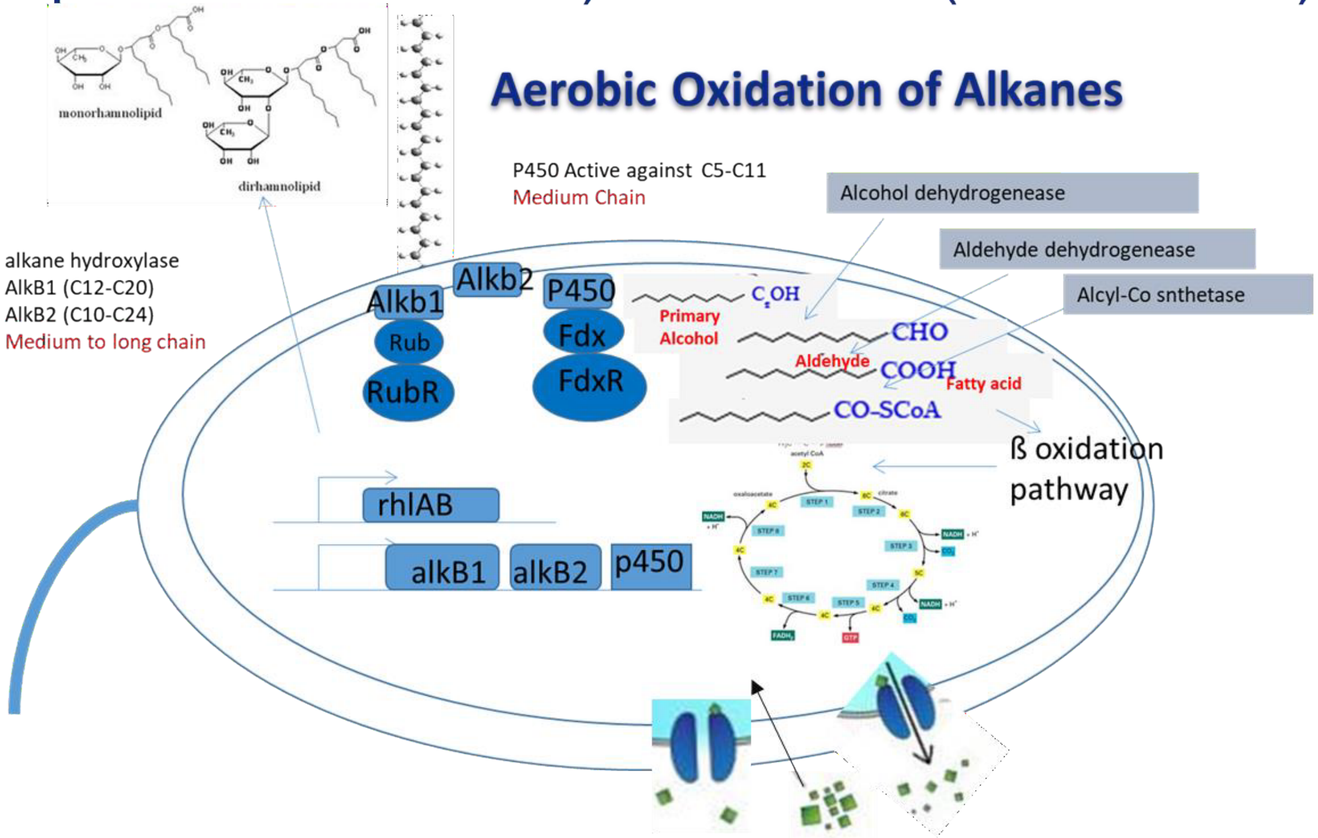
Without iron

With iron



Microbial Mechanisms of Adaptation to Hydrocarbons

Survival in Fuel (Adaptive Mechanisms) ≠ Fuel Degradation (Metabolism)



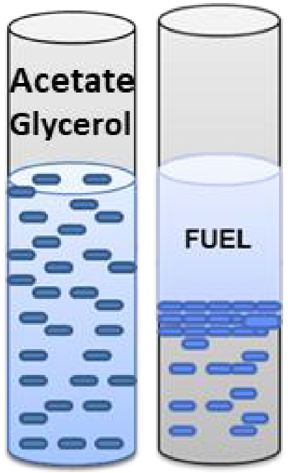
OMICS for Understanding Fuel Biodeterioration

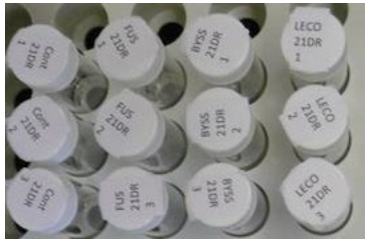
Approach:
- Use of microbiology for isolation and growth of strains
- GC-MS for determination of hydrocarbon degradation profile
- DNA Seq for microbial identification/microbiome profiling
- RNA seq for gene expression profiling
- LC-MS for metabolomics & proteomics for defining metabolic activity
- Functional assays to confirm Omics results

Isolation, Identification, Quantification

Chemical Analysis

Growth Bioassays

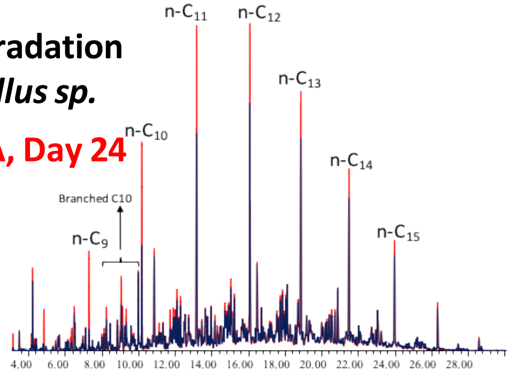




Gas Chromatography-Mass Spectrometry

Hydrocarbon degradation profile of *Aspergillus sp.*

Neg. control Jet A, Day 24

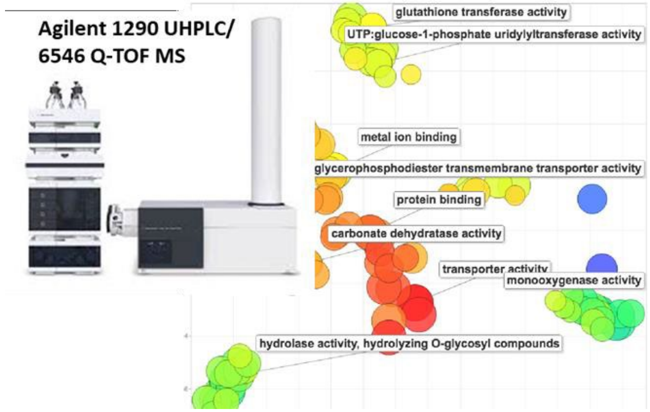


Genomics & Transcriptomics

Illumina Hi Seq DNA Sequencer plus Bioinformatics

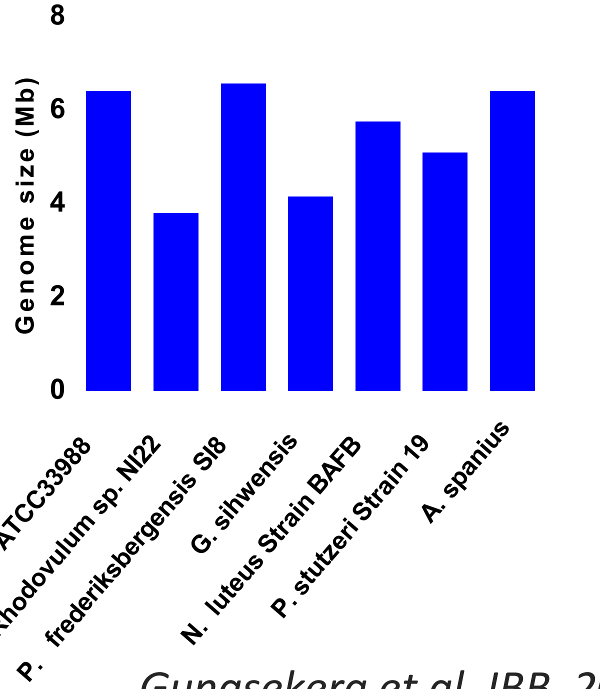
Proteomics & Metabolomics

Agilent 1290 UHPLC/ 6546 Q-TOF MS

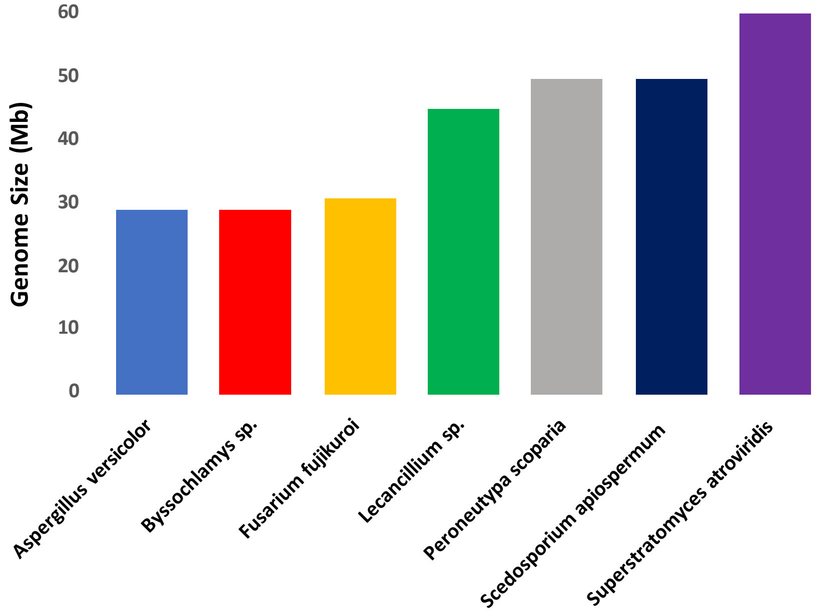


Genomics

Bacterial Genomes Sequenced



Fungal Genomes Sequenced



Results

- 20 bacterial genomes sequenced/annotated
- 17 fungal genomes sequenced/annotated (>10X larger than bacterial genomes)

Brown et al., Genome A 2014, 2015,2016, 2017(a)(b)
Ruiz et al. Genome A, 2015
Radwan et al. Genome A, 2018 (a)(b)
Gunasekera et al. MRA, 2018
Radwan et al. MRA, 2019

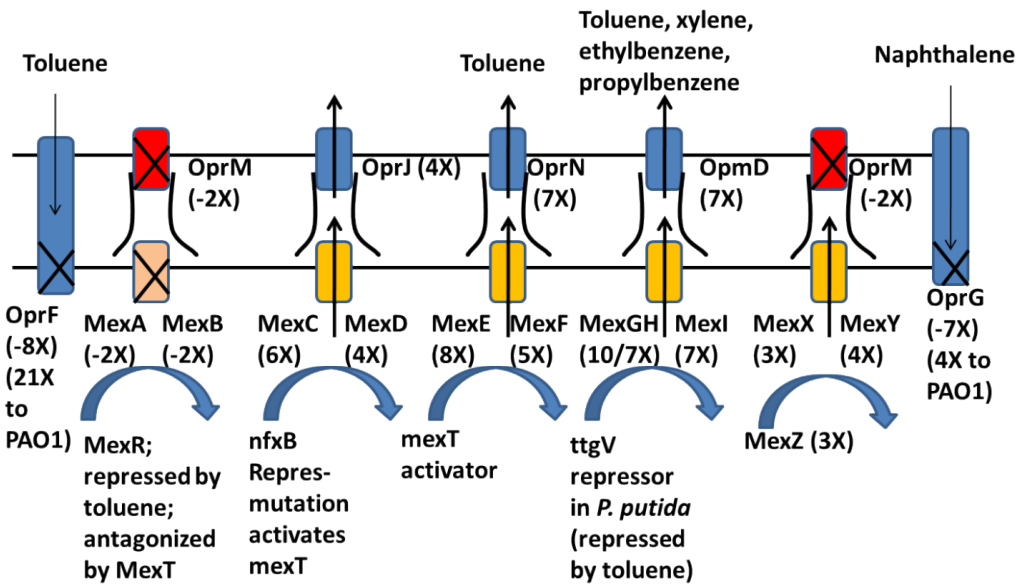
Gunasekera et al. IBB, 2022
Ruiz & Radwan, MRA, 2021
Radwan & Ruiz, MRA, 2021(a)(b)
Ruiz et al., Data in Brief, 2021
Ruiz et al., IBB, 2020
Grady et al. BMC Genomics, 2017

Gunasekera et al., AEM, 2017
Ruiz et al., Energy & Fuels, 2016
Ruiz et al., Genome A, 2015
Striebich et al.,IBB, 2014
Gunasekera et al., Env Sci Technol, 2013

Mechanisms for Fuel Adaptation in *P. aeruginosa* 33988

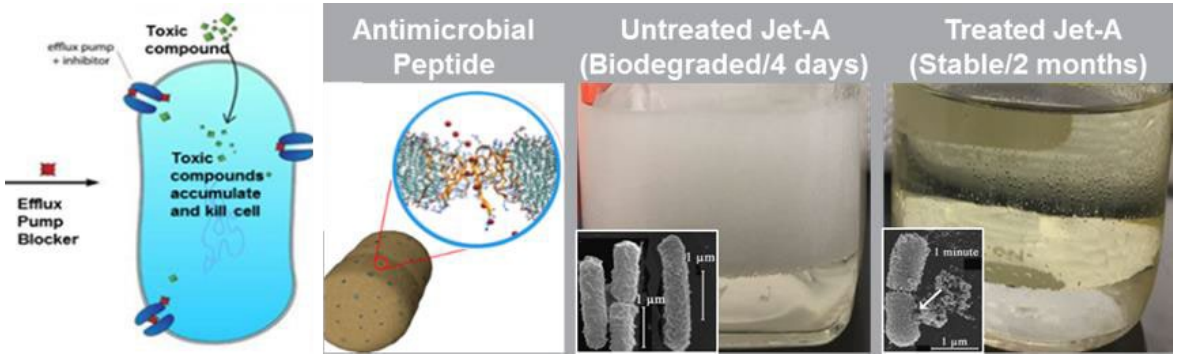
1. **Pathways related to terminal oxidation of alkanes (oxygenases, P450)**
2. **Secretion of extracellular polysaccharides (alginate) and rhamnolipids, biofilm formation**
3. **Regulation of efflux pumps and porins**
4. **Iron transport and metabolism**
5. **Stress response and quorum sensing**

Membrane Transporters Regulation



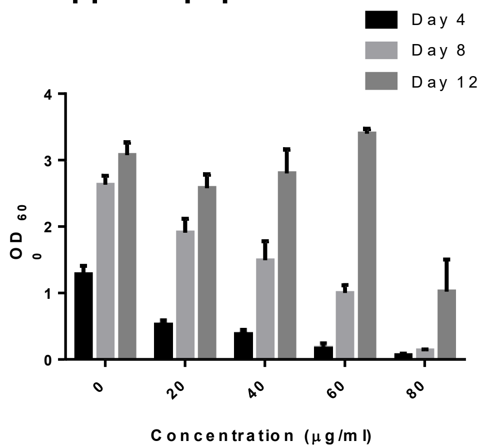
Gunasekera et al., 2013 Environ. Sci. Technol. 2013, 47, 13449−13458

Advanced Fuel Biocide (AFB)

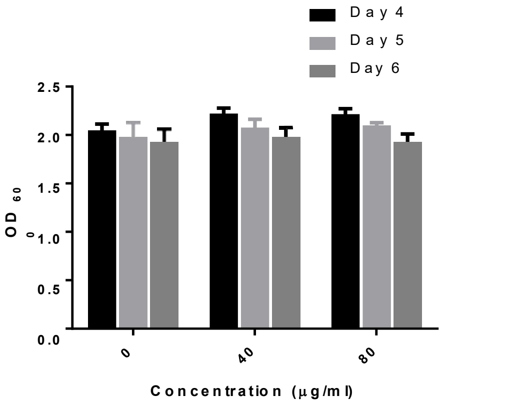


Defeats microbial resistance mechanisms, non-toxic to people, patented

C-capped dipeptide in Fuel

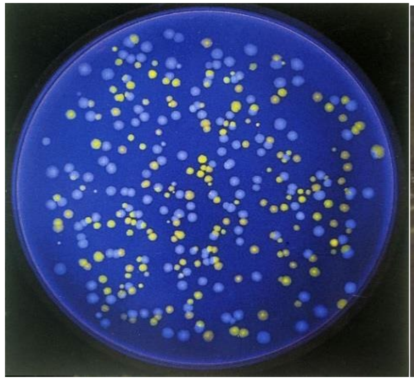
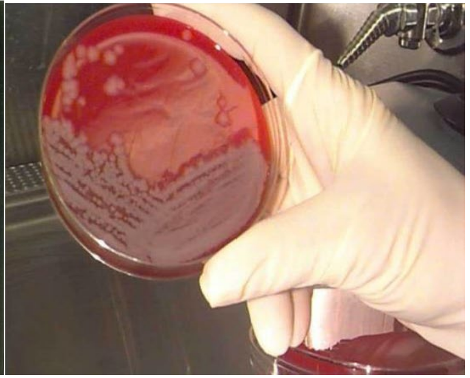
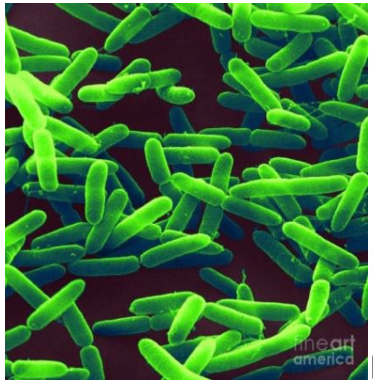


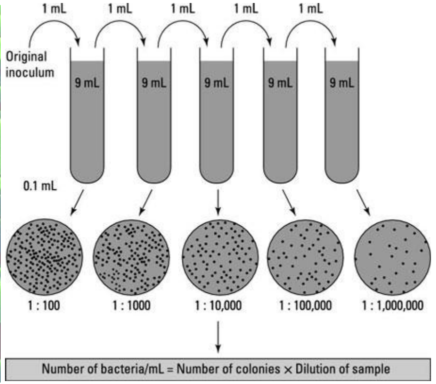
C-capped dipeptide in glycerol



Efflux pump inhibitor: C-capped dipeptide Phe-Arg-β-Naphthylamide dihydrochloride (MC-207)

ASTM Standard Test Methods

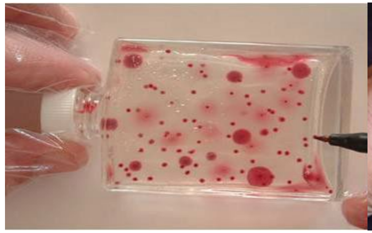






- ASTM 6974-09 Standard Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels- Filtration and Culture Procedure. ***(Per IATA: High Contamination above 20,000 CFU/L fuel or 10,000 CFU/mL water)***

- ASTM D7463 Test method for Adenosine Triphospahate (ATP) content of Microorganisms in Fuel, Fuel/Water Mixtures and Fuel associated Water. ***(High Contamination above 1,000 RLU)***

- ASTM D8070: Standard Test Method for Screening of Fuels and Fuel Associated Aqueous Specimens for Microbial Contamination by Lateral Flow Immunoassay


MicrobMonitor


Bug Alert kits

FuelStat kits

HY-liTe

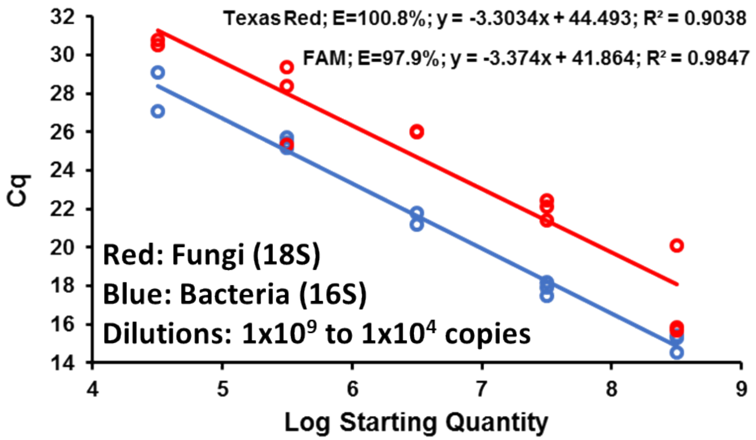
ASTM Resorces

- D6469-17: Standard Guide for Microbial Contamination in Fuels and Fuel Systems
- D7464-14: Standard Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing
- ASTM 6974-09 Standard Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels- Filtration and Culture Procedure.
- ASTM D7463 Standard Test method for Adenosine Triphospahate (ATP) Content of Microorganisms in Fuel, Fuel/Water Mixtures and Fuel associated Water.
- ASTM 7687-11 Standard Test Method for Measurement of Cellular Adenosine Triphosphate in Fuel, Fuel/Water Mixtures, and Fuel Associated Water with Sample Concentration by Filtration.
- ASTM D8070: Standard Test Method for Screening of Fuels and Fuel Associated Aqueous Specimens for Microbial Contamination by Lateral Flow Immunoassay
- ASTM D8243: Standard Test Method Determination of APS Reductase to Estimate Sulfate Reducing Bacterial Bioburdens in Water – Enzyme-Linked Immunosorbent Assay Method
- ASTM D8412-21: Quantification of Microbial Contamination in Liquid Fuels and Fuel-Associated Water by Quantitative Polymerase Chain Reaction (qPCR)

qPCR Assay for Rapid Detection of Microbes in Fuel Based on DNA Detection

Impact / Importance:

- Rapid detection of fuel bioconamination (~30 Min)
- AFRL developed technology
- First-ever international standard for DNA-Based detection of fuel microbial contamination
- Working toward implementation with DoD Fuel Area Labs



- Radwan, O., Gunasekera, T.S. and Ruiz, O.N. 2018. Robust Multiplex Quantitative Polymerase Chain Reaction Assay for Universal Detection of Microorganisms in Fuel. *Energy & Fuels*. DOI: 10.1021/acs.energyfuels.8b02292

This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

ASTM INTERNATIONAL

Designation: D8412 – 21

Standard Guide for Quantification of Microbial Contamination in Liquid Fuels and Fuel-Associated Water by Quantitative Polymerase Chain Reaction (qPCR)[1]

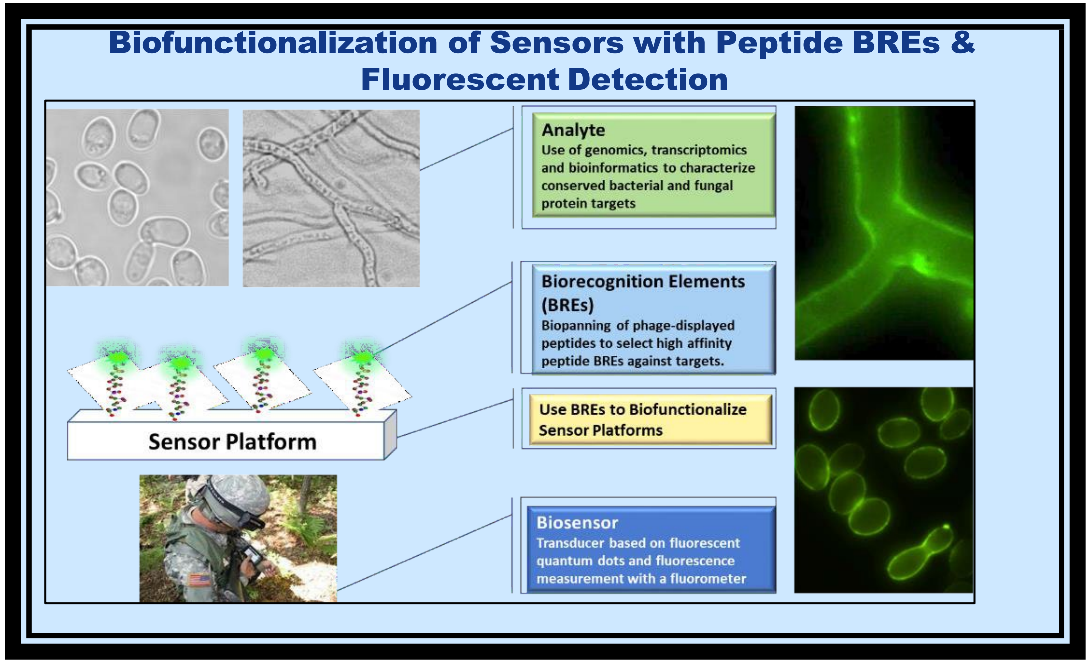
This standard is issued under the fixed designation D8412; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

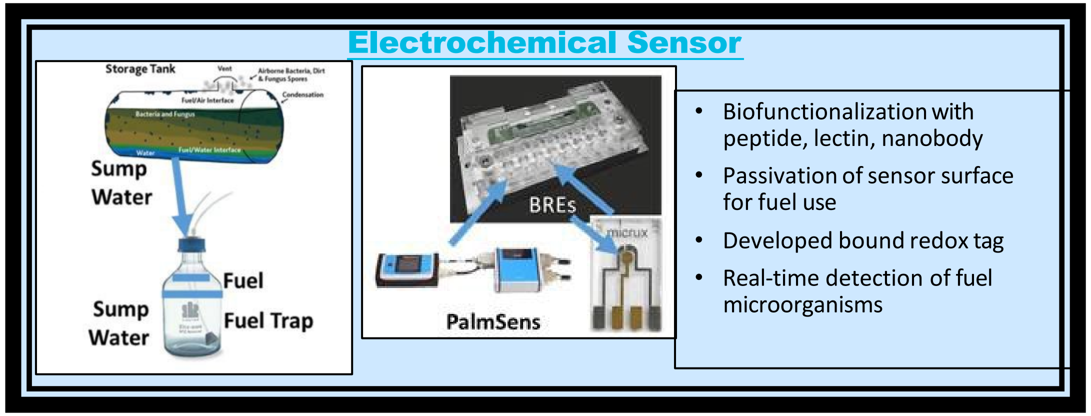
https://www.luminultra.com/tech/genecount-qpcr/

Biosensors for Rapid Detection of Fuel Biocontamination

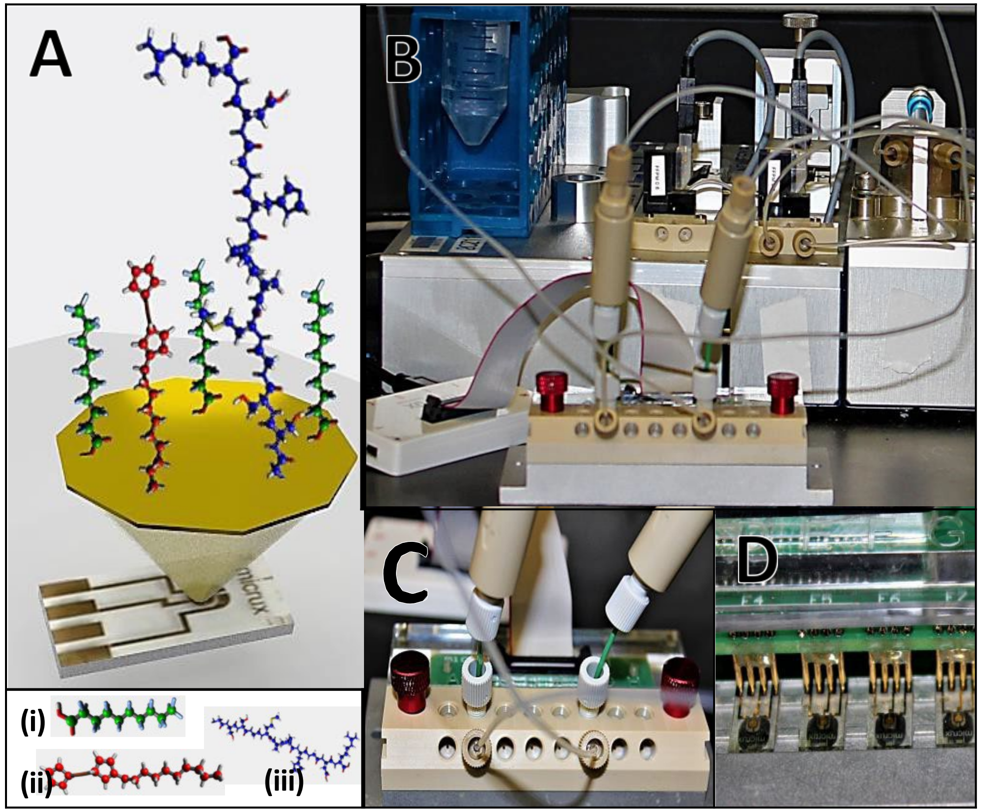
Impact / Importance:

- Rapid detection & quantification of fuel biocontamination
- Broad detection & differentiation of microbial biocontaminats
- Fluorescent, photonics and electro-chemical sensors
- IP based on foundational Omics research
  - U.S. Patent 10,295,537. Biorecognition elements for rapid detection of biocontamination
  - U.S. Patent 11,754,547. Biorecognition elements for detection of fungi and bacteria in fuel systems
- Radwan, O., Brothers, M.C., Coyle, V., Chapleau, M.E., Chapleau, R.R., Kim, S.S. and Ruiz, O.N., 2022. Electrochemical biosensor for rapid detection of fungal contamination in fuel systems. *Biosensors and Bioelectronics*, p.114374.
- Radwan et al. 2023. Discovery, development and implementation of biomarker-specific peptide BRE for biosensing hydrocarbon-degrading fungi, Int Biodegr Biodeter.
- Pavlyuk and Ruiz. 2017. Peptide-based fluorescent biosensing for rapid detection of fuel biocontamination, Energy & Fuels.





Detection of Fuel Fungal Contamination with Electrochemical Sensor



(A) Schematic of a commercially available Micrux Chip functionalized by the reported SAM including (i) 10-chain fluorinated carbon (green) (ii) ferrocene redox reporter (red) and (iii) the chitinase BRE (blue). (B-D) Experimental set-up of the Micrux chip interfaced with the Micrux flow cell (8-channel) microfluidic cell system

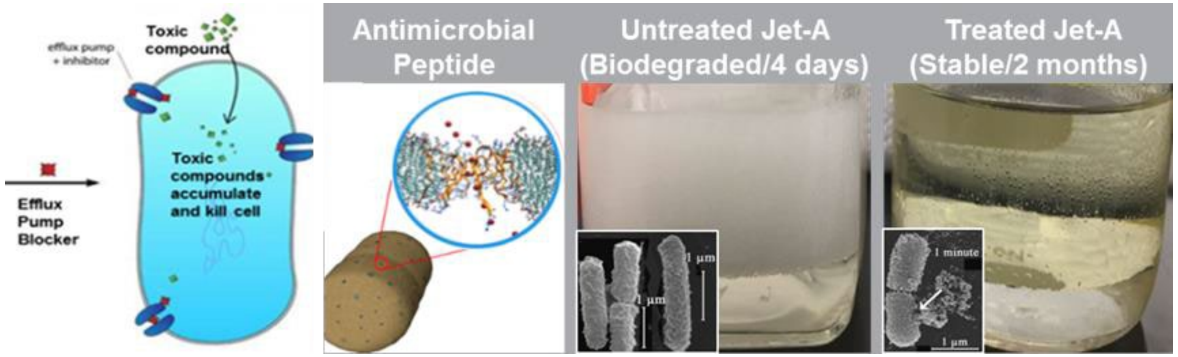
FY 20-21Deliverables/Milestones/Accomplishments

Description: Advanced Fuel Biocide (AFB) provides effective microbial growth prevention while being non-toxic to people and the environment.

Impact / Importance:

- Military, OEMs and airlines are in desperate need of a new effective biocide to prevent fuel system biodeterioration. The main commercial broad-spectrum fuel biocide has been recently banned for aviation
- Based on RQTF foundational work, AFB uses two active ingredients targeting specific functions of fuel-degrading microbes.
- First-of-its kind, acts as a medicine for the fuel system
- AFB is applied at ultra-low concentration to the fuel.
- Current at TRL- 5.
- U.S. Patents 10,487,281; 10,876,059; 10,876,059. Method for prevention of biodeterioration of fuels
- Development & commercialization CRADAs
- AFB passed fuel spec testing & long duration effect
- PAYOFF: The AFB will reduce sustainment cost, and increase mission readiness, operability & capability. Hugh commercial market.

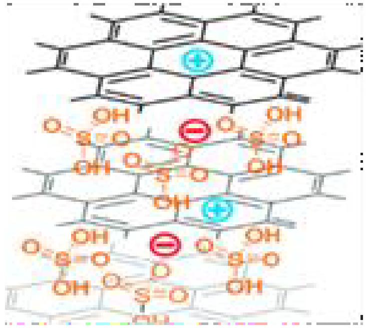
Advanced Fuel Biocide (AFB)

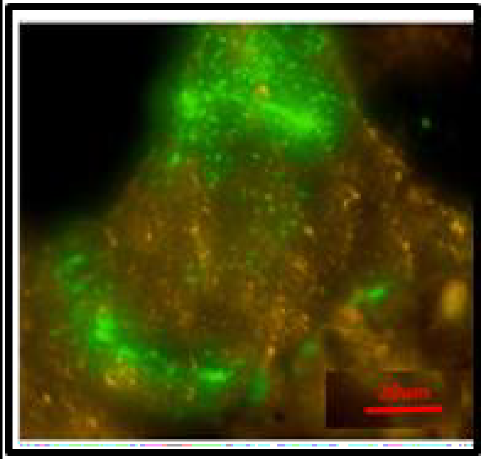


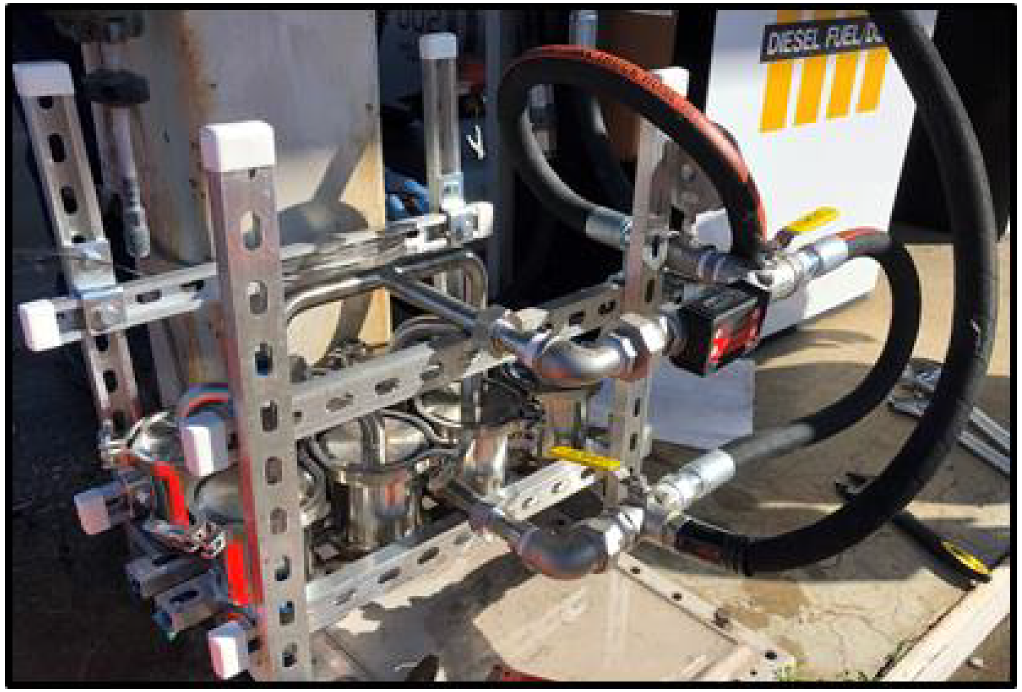
Defeats microbial resistance mechanisms, non-toxic to people, patented

Development and Transition Plan to TRL-9:
currently in conversations with OEMs and additive manufacturers to support tasks.
Task 1: Field test demonstration in small and medium size fuel tanks (TRL-7,Yr 1)
Task 2: Perform fuel system/engine & material compatibility tests (TRL-8, Yrs 1-2)
Task 3: Obtain EPA approval & ASTM certification (aircraft use) (TRL-9, Yrs 1-3)
Task 4: Secure large-scale manufacturing sources (Yrs 1-2)

Microbial Fuel Filter







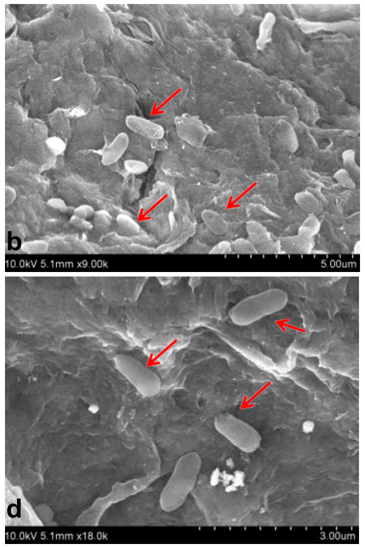
99.9% microbial filtration, First-ever nano-based technology for microbial filtration, **not** dependent on small pores filters, modular, TRL-7, patented

Graphene Oxide-Based Microbial Fuel Filter

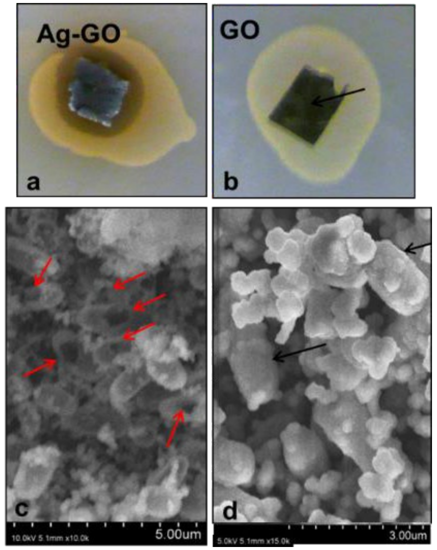
ACCOMPLISHMENTS:

- Nanofiltration media removes more than 99.97% of all microbes in jet fuel while allowing rapid fuel flow.
- Graphene oxide was coated with nano-silver to increase antimicrobial activity, the filter's efficiency and service life
- *U.S. Patent* 9403112 (Issued 2 Aug 2016): *"GRAPHENE OXIDE FILTERS AND METHODS OF USE"*
- *Ruiz et al. 2015. Int. Biodeter. Biodegr., vol 95, 168-178:* Graphene oxide-based nanofilters efficiently remove bacteria from fuel.
- *Ruiz et al. 2013. ACS Nano, 5, 8100-8107.*
- Partnership with companies to develop AFRL fuel nanofiltration technology

SEM of Bacteria Trapped by Nanofilter

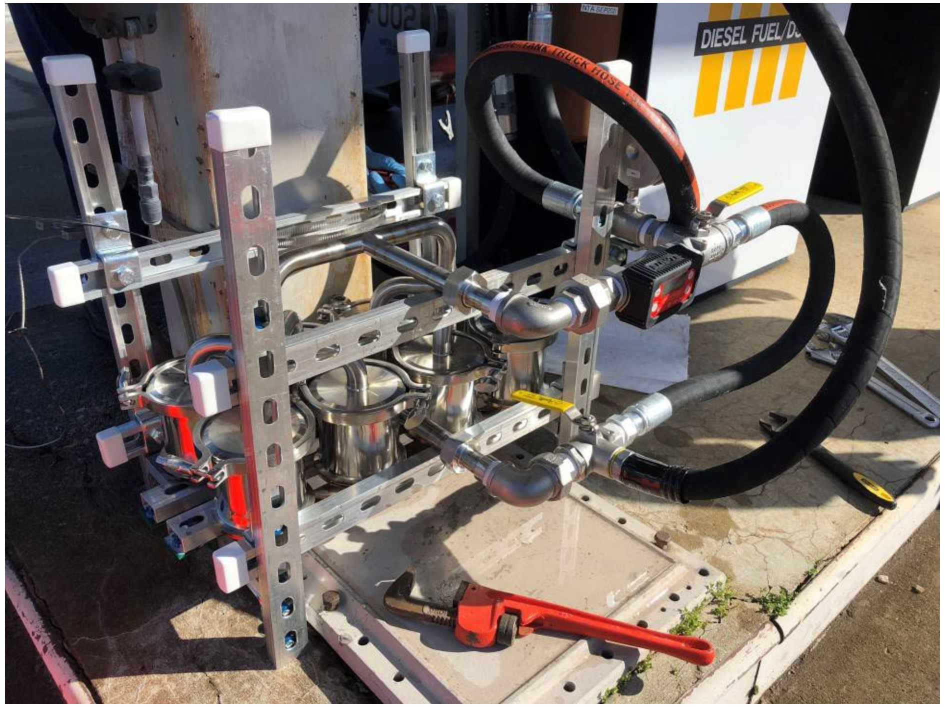


Antimicrobial Ag-GO (a,c)



Arrows indicate bacteria cells on the nanomaterial matrix

Field Demo– 20 GPM DS1 Pump



- No Pressure drop!
- Initial rate w/o filter 17.5 gal/min
- Rate w/ filter 17.5 gal/min
- In the lab, 100K gallons of diesel fuel filtered without changes in fuel spec properties.
- >90% microbial filtration efficiency over filter lifetime.
- In the field, people are excited about this filter as more biofuels are being used in the future.
- TRL-7, meets ASTM D975, scalable

Generator Field Assessment

- ❑ Installed a GO filter suitable for 0.3 LPM flow rate in a 18 kW generator
- ✓ Operated generator connected to a 30 kW load bank with a load cycle of 3 kW (17%) and 13 kW (72%)
- ✓ No noticeable difference observed operating the generator with and without filter

Recommendations for Dealing with Tank Biocontamination

1. When possible, biocide the tank following manufacturer instructions but providing the longest contact time allowed to penetrate biofilms in surfaces and interface.

2. Drain the sump until no water or biological material flows.

3. Optimally:
   - The fuel drained from the sump as well as the remainder in the tank can be polished/filtered to remove water and particulates.
   - During the polishing process the fuel should be directed into a clean tank.
   - When the tank inspection confirms that all biological material sludge and water has been removed the polished fuel can be returned to the original tank.
   - Replace all filters used to polish the contaminated fuel.

4. An additional dose of biocide may be applied to the tank.

5. Continue to aggressively sump the tank to ensure that water does not build up.

Note: If biocide cannot be used, the contaminated tank should be thoroughly clean by pressure washing, scooping sludge, and drying before any new fuel is added.

