Investigation of relationship between water content in biodiesels and microbial growth and contamination

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Background

- **Distillate fuels containing Fatty Acid Methyl Esters (FAME) are more prone to microbial growth.**

- **Previous Energy Institute (EI) laboratory study;**
  - Diesel containing ≥2% FAME more microbial growth (biomass) and faster growth.

Background

- **Biofuels containing FAME have increased propensity to hold water.**
  - Potential for microbial growth throughout the bulk fuel in storage tanks?
  - Impede the ability of microbial contamination to be removed by routine tank settling?

- **2015 EI laboratory study**
  - Further work to investigate the relationship between water content and microbial growth in biodiesels.

Project Overview

- **Part 1**: Investigating the influence of FAME concentration and total water content on;
  - overall microbial growth.
  - the distribution of water and microbes in fuel phase over a 14 week period.

- **Part 2**: Investigating the influence of settling time on the vertical distribution of microbial contamination and water;
  - Selected microcosms shaken vigorously at the end of Part 1.
  - Settling of water and microbes monitored over a 48 h period.

PART 1
Microcosm set up

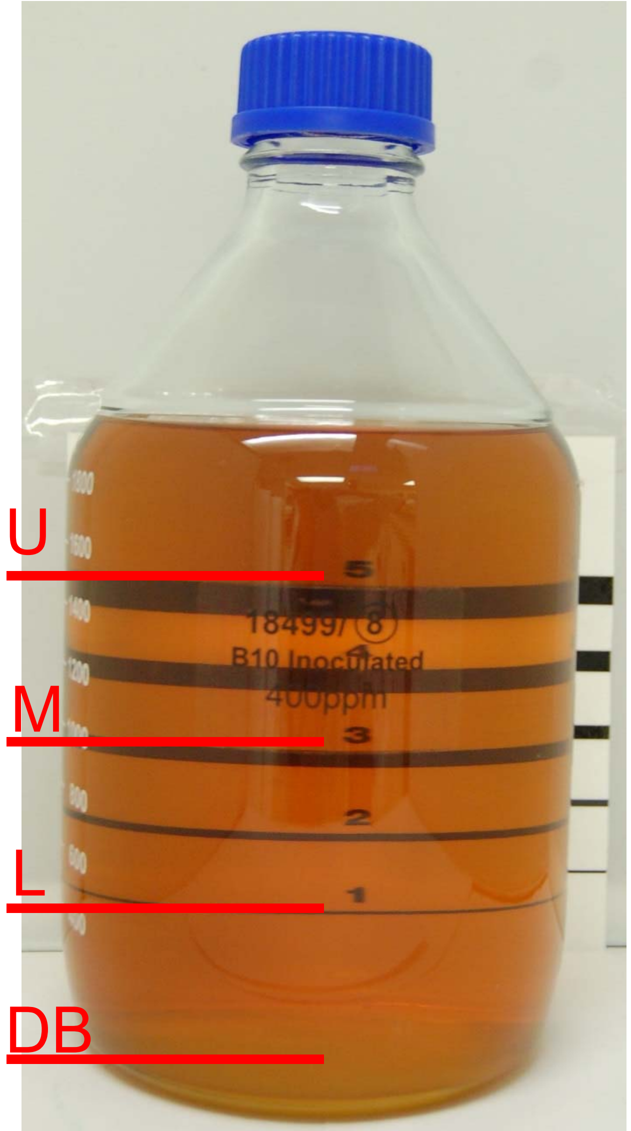
- 2 litre fuel microcosms containing biodiesel blends
  - B0, B10 and B20
- Held at 21°C & 70% RH in an environmental chamber.
  - Allowed to equilibrate.
  - Water content measured and then TOTAL water content adjusted to desired levels of 100 ppm, 400 ppm, 1000 ppm and 10,000 ppm.
  - Depending on water holding capacity of fuel this would be present as free and/or dissolved water.
- Water added included low numbers (c. 800 CFU) of known fuel degrading microorganisms and isolates from a variety of field samples.
  - Range of bacteria (11), yeasts (9) and moulds (9).
  - Un-inoculated microcosms also set up as a control.

PART 1
Microcosm set up

	Fuel Blend					
TOTAL Water Concentration (ppm)	B0		B10		B20	
	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated
100	✓	✓	✓	✓	✓	✓
400	✓	✗	✓	✗	✓	✗
1000	✓	✗	✓	✗	✓	✗
10000	✓	✓	✓	✓	✓	✓

PART 1 Assessments

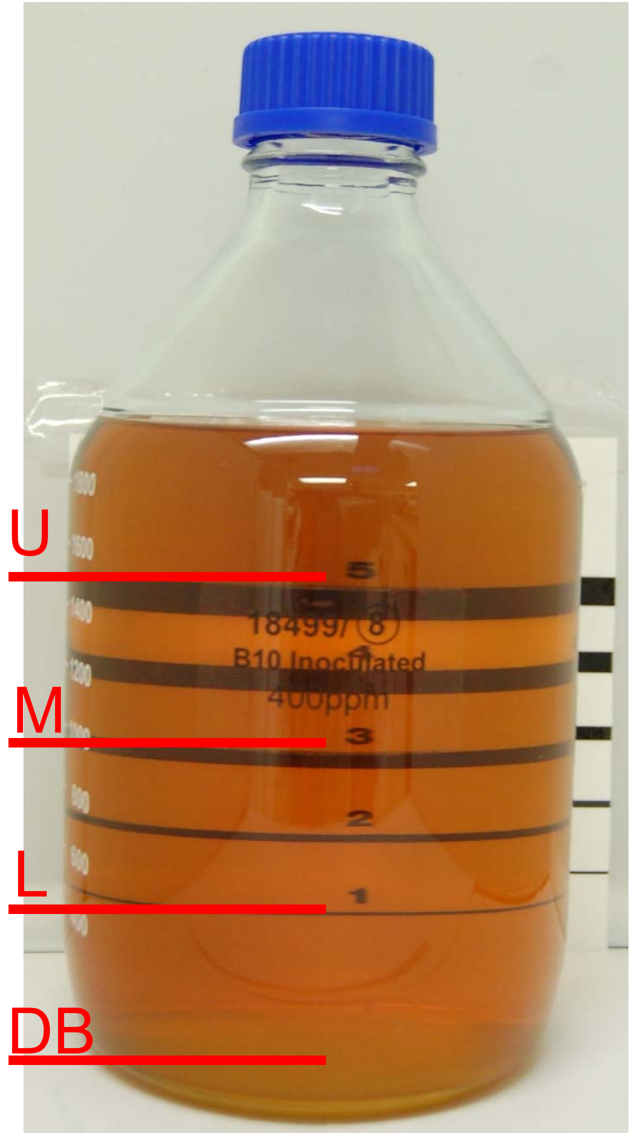
- Visual assessment every week.
- Each microcosm gently agitated after visual assessment (and sampling as required).
- Fuel sampled after 1, 2, 4 and 14 weeks at 4 depths;
  - Upper layer, middle, lower and dead bottom (above any visible aqueous phase or biomass).
  - Total Viable Count (TVC) by IP 613/14 (ASTM D7978-14).
  - Water content by Karl Fischer IP 438/01.



PART 1 Assessments

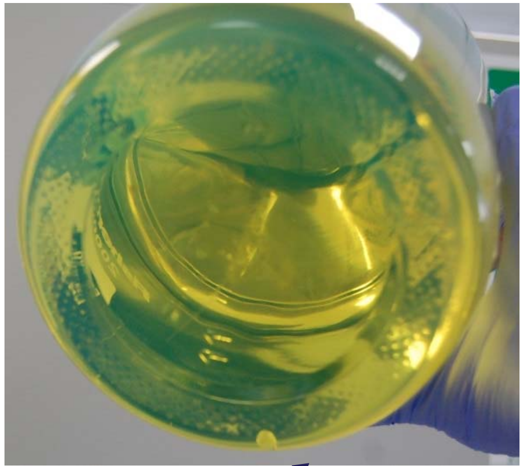
Additional assessments at end of trial

- Fuel sampled from the middle layer.
  - Total Acid Number (TAN) by IP 139/98.
  - Filter Blocking Tendency (FBT) by IP 387/14.
- Aqueous phase (if sufficient present)
  - TVC of bacteria, yeasts and moulds.
- Bottom 800 ml (including aqueous phase and any biomass present);
  - Filterable particulate content by modified IP 415/07.

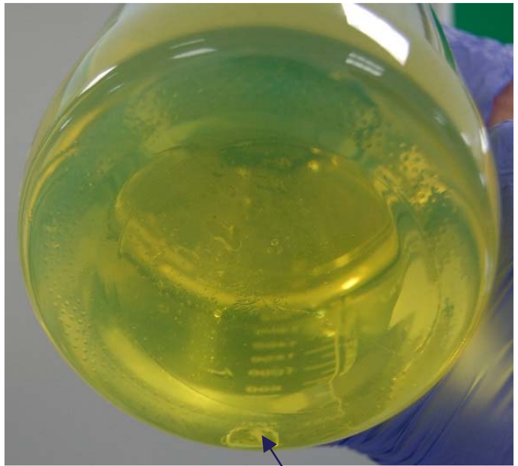


B0 Microcosms at week 14

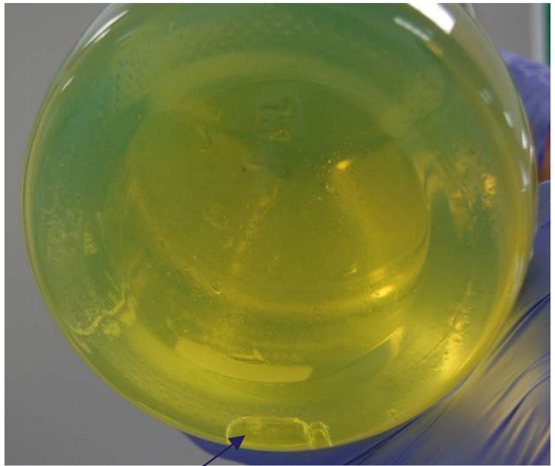
100 ppm



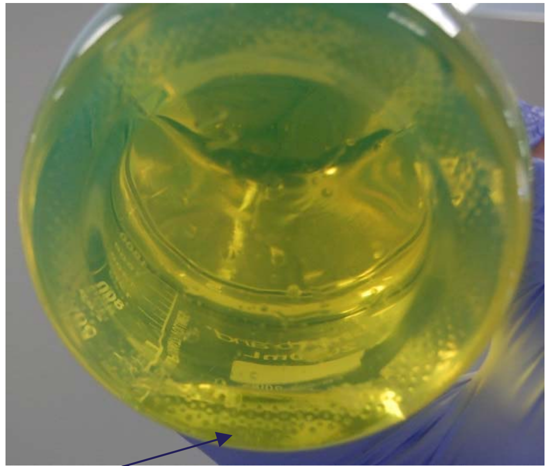
400 ppm



1000 ppm



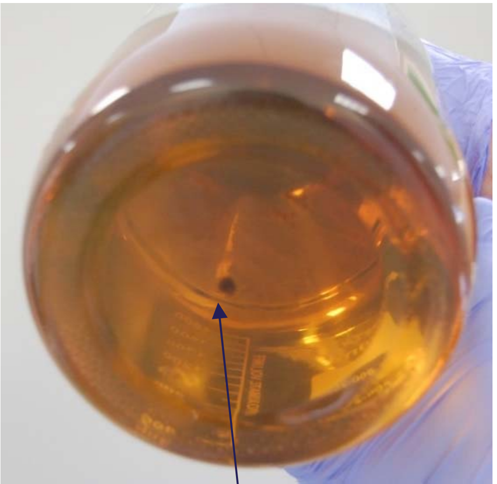
10 000 ppm



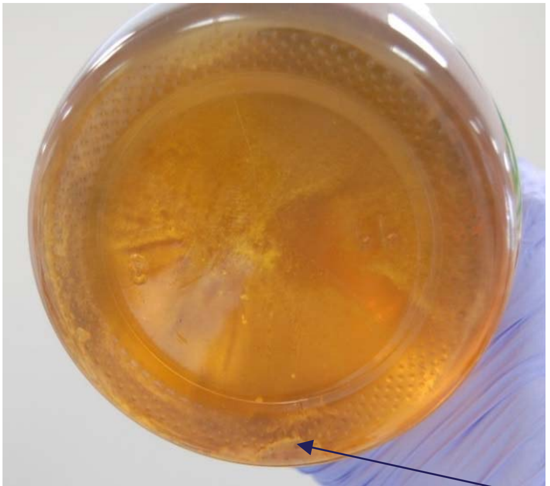
Turbid water containing white floccose biomass

B10 Microcosms at week 14

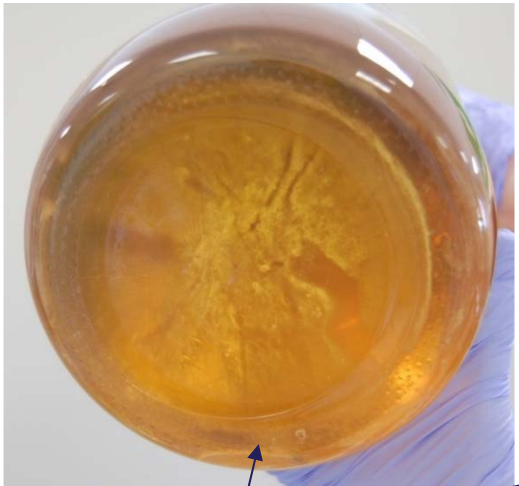
100 ppm



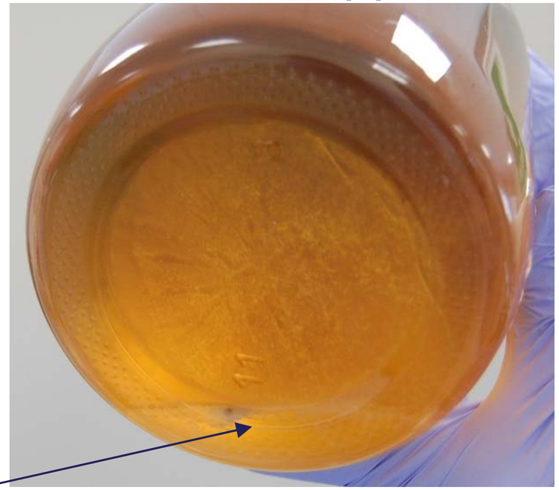
400 ppm



1000 ppm



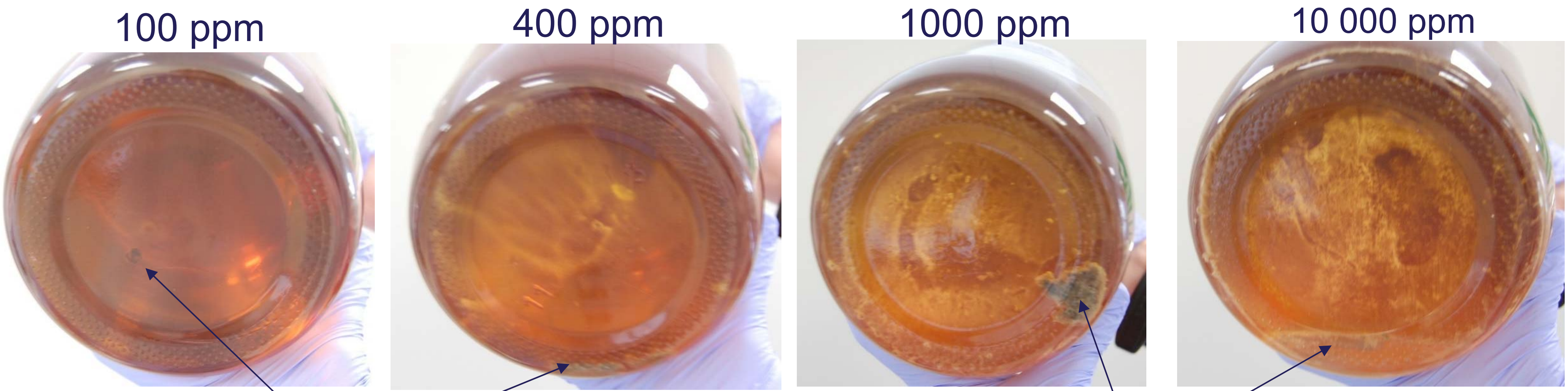
10 000 ppm



Lump of brown biomass;
no visible free water

Turbid water phase containing white and brown floccose biomass

B20 Microcosms at week 14

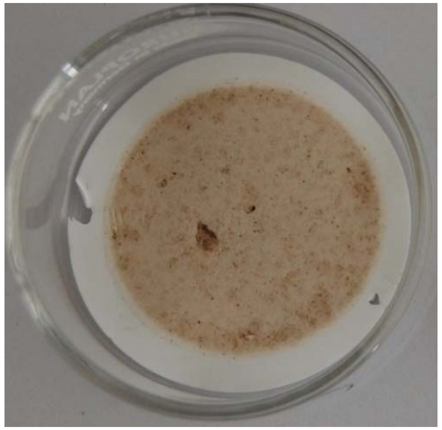
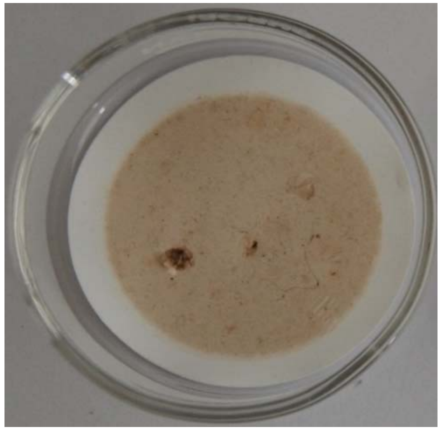
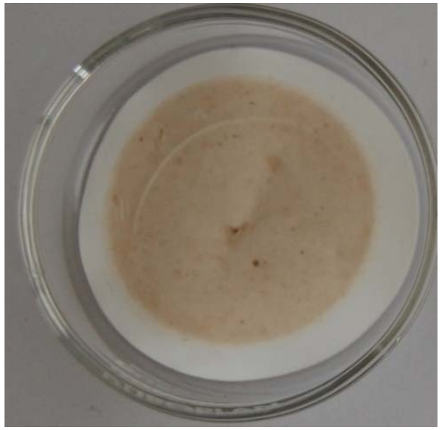


Lumps of grey-brown biomass;
No visible water phase

Turbid water phase.
Lumps of grey-brown biomass

- **More microbial biomass accumulating at the bottom of the microcosms with increasing FAME concentration**

Modified IP415: biomass collected on filters

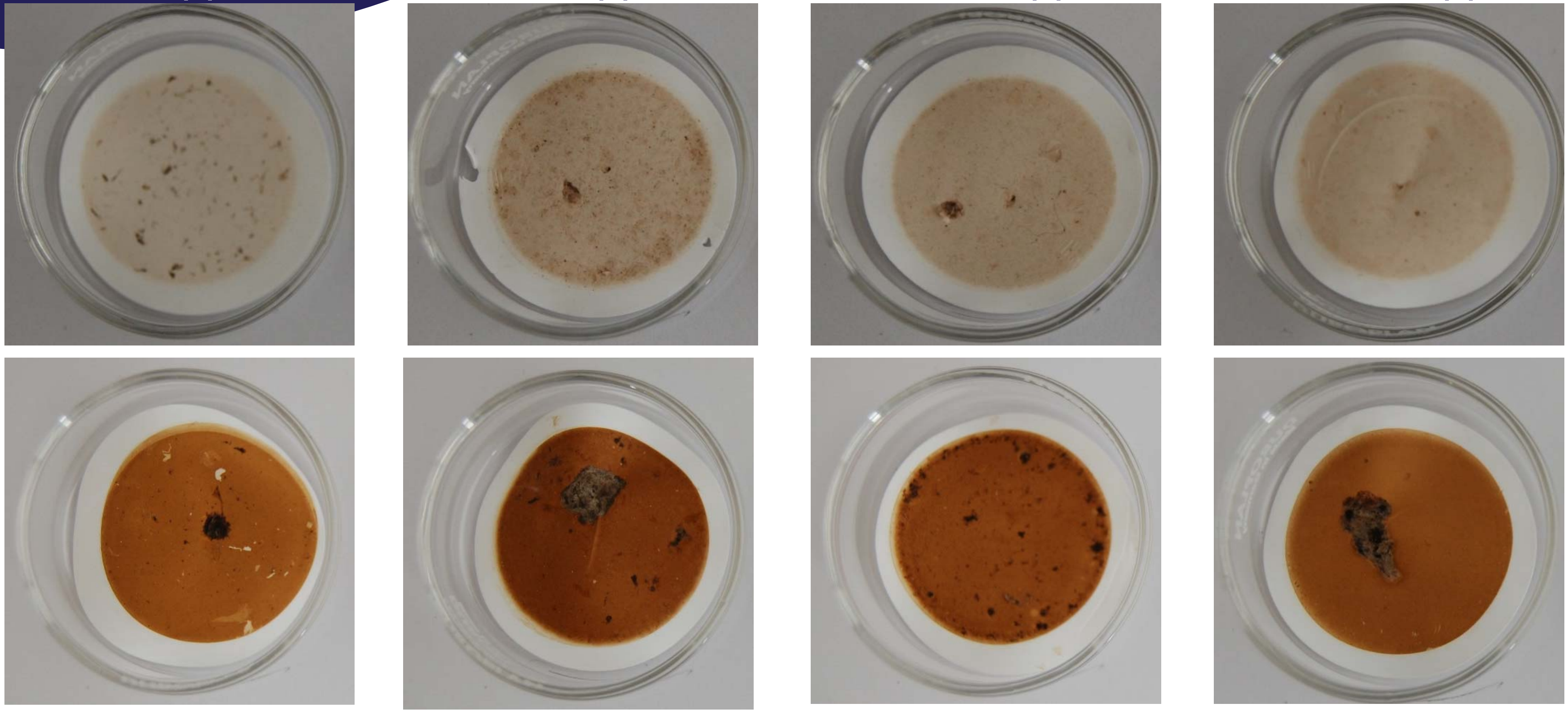
	100 ppm	400 ppm	1000 ppm	10 000 ppm
B0				

Modified IP415: biomass collected on filters

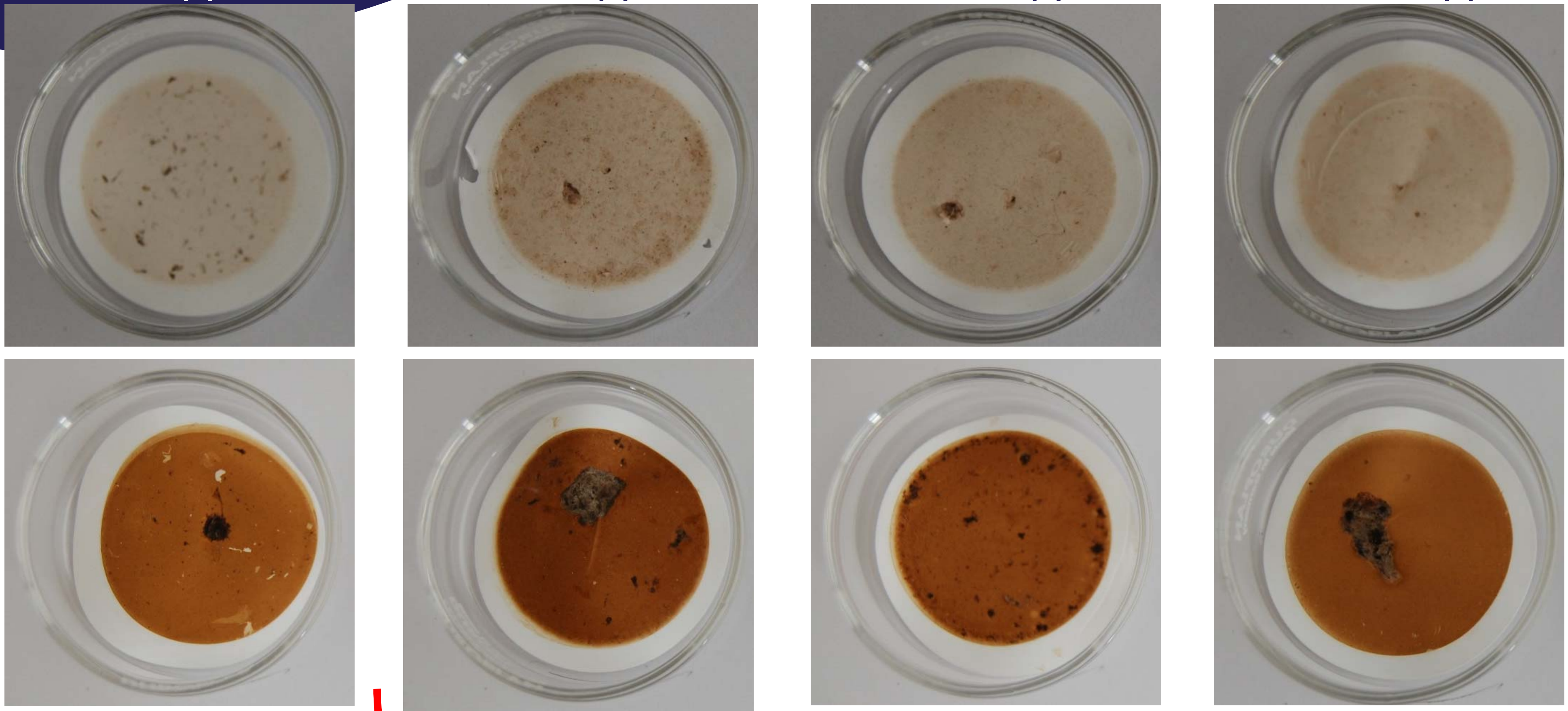
100 ppm | 400 ppm | 1000 ppm | 10 000 ppm

B0

B10

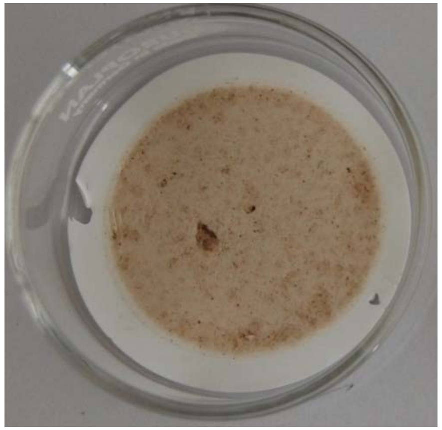
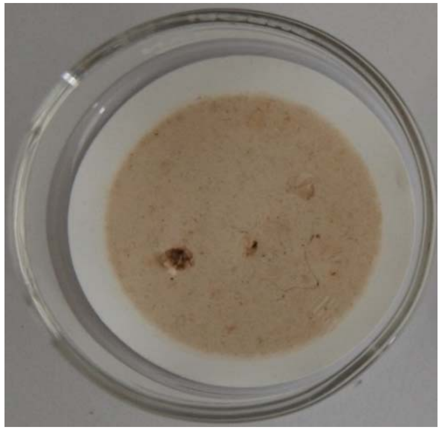


Modified IP415: biomass collected on filters



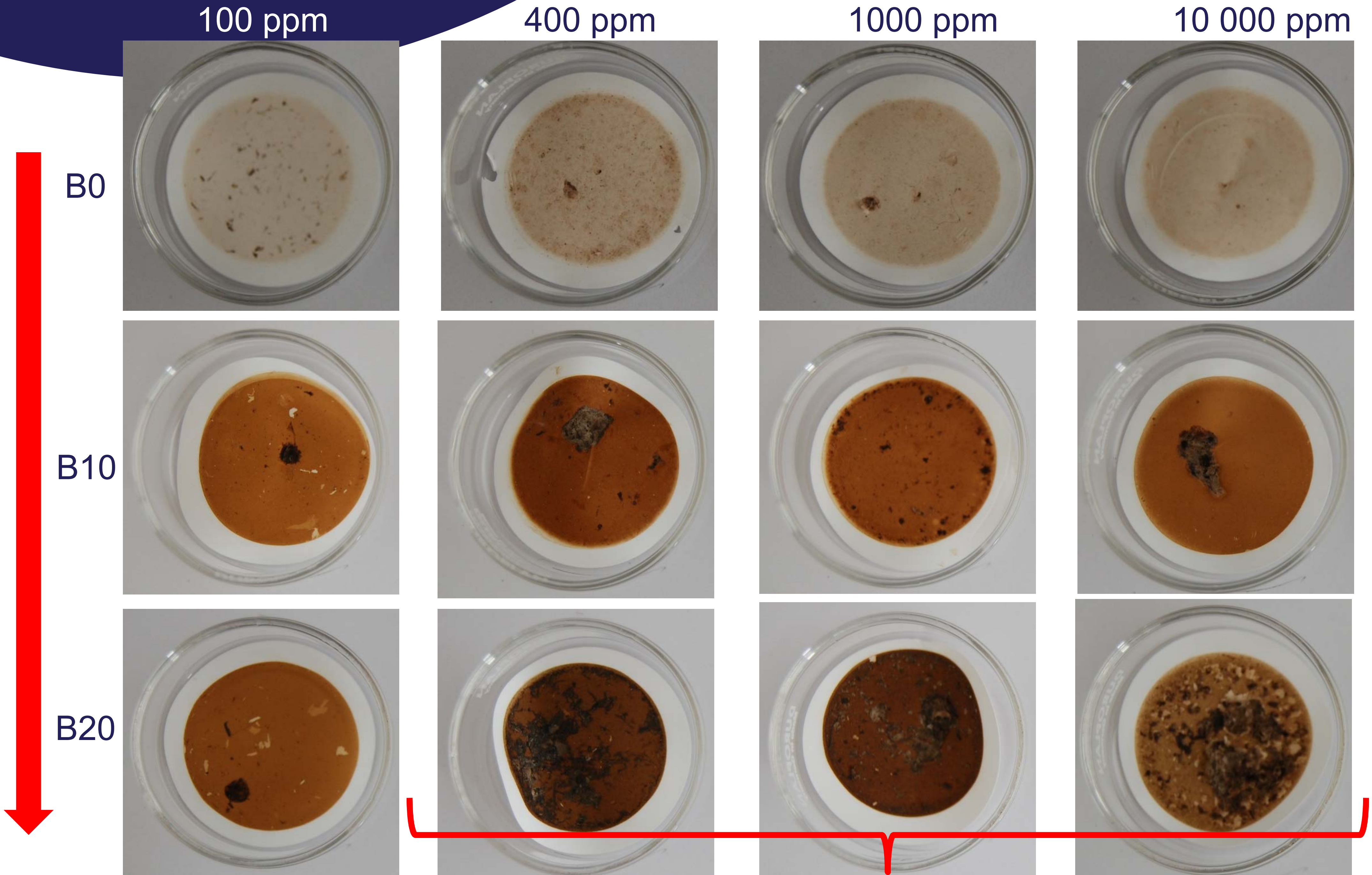
- **Evidence of more microbial biomass accumulating with increasing FAME content (particularly in microcosms containing ≥400 ppm total water)**

Modified IP415: biomass collected on filters

	100 ppm	400 ppm	1000 ppm	10 000 ppm
B0				
B10				
B20				

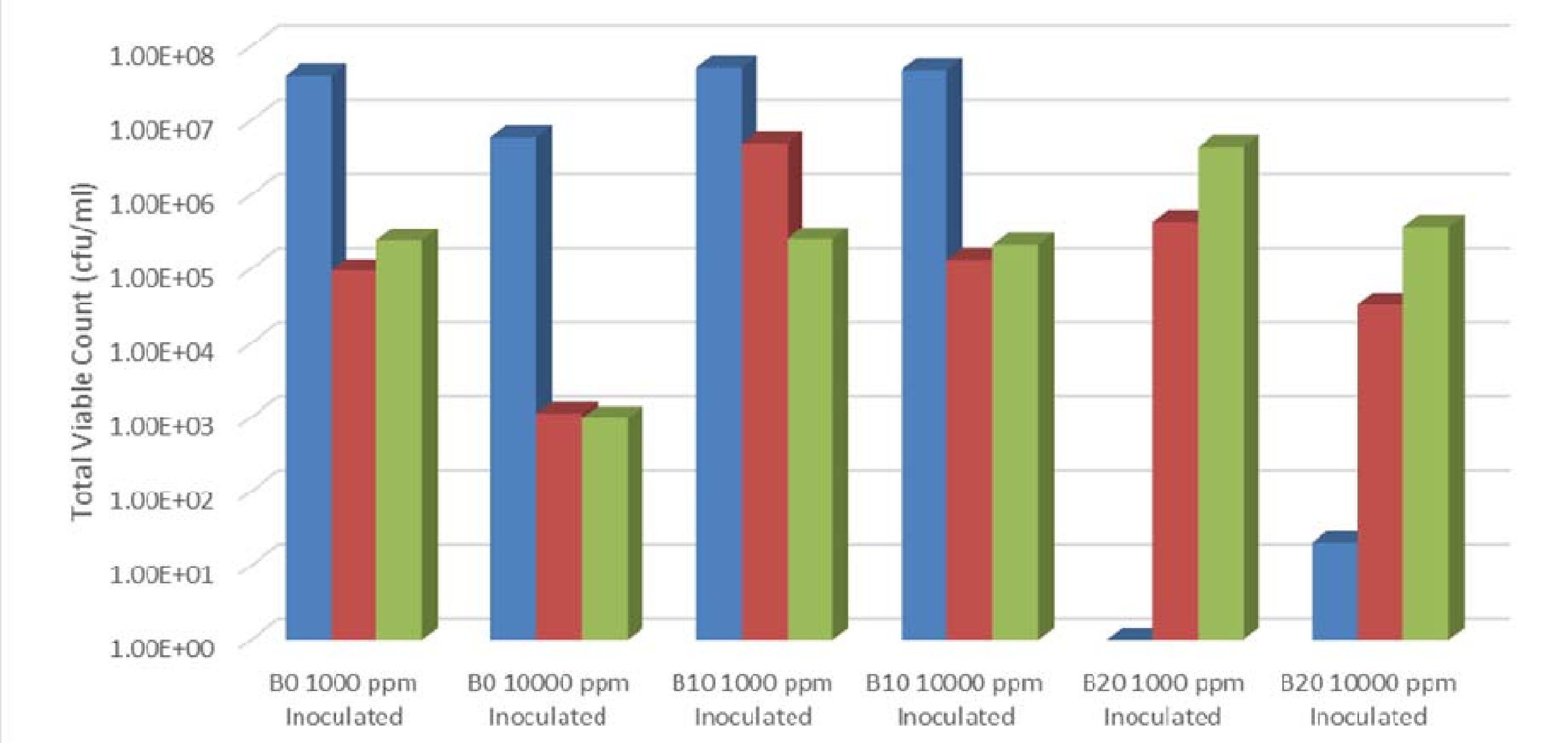
Modified IP415: biomass collected on filters

energy institute



Microbes in the aqueous phase

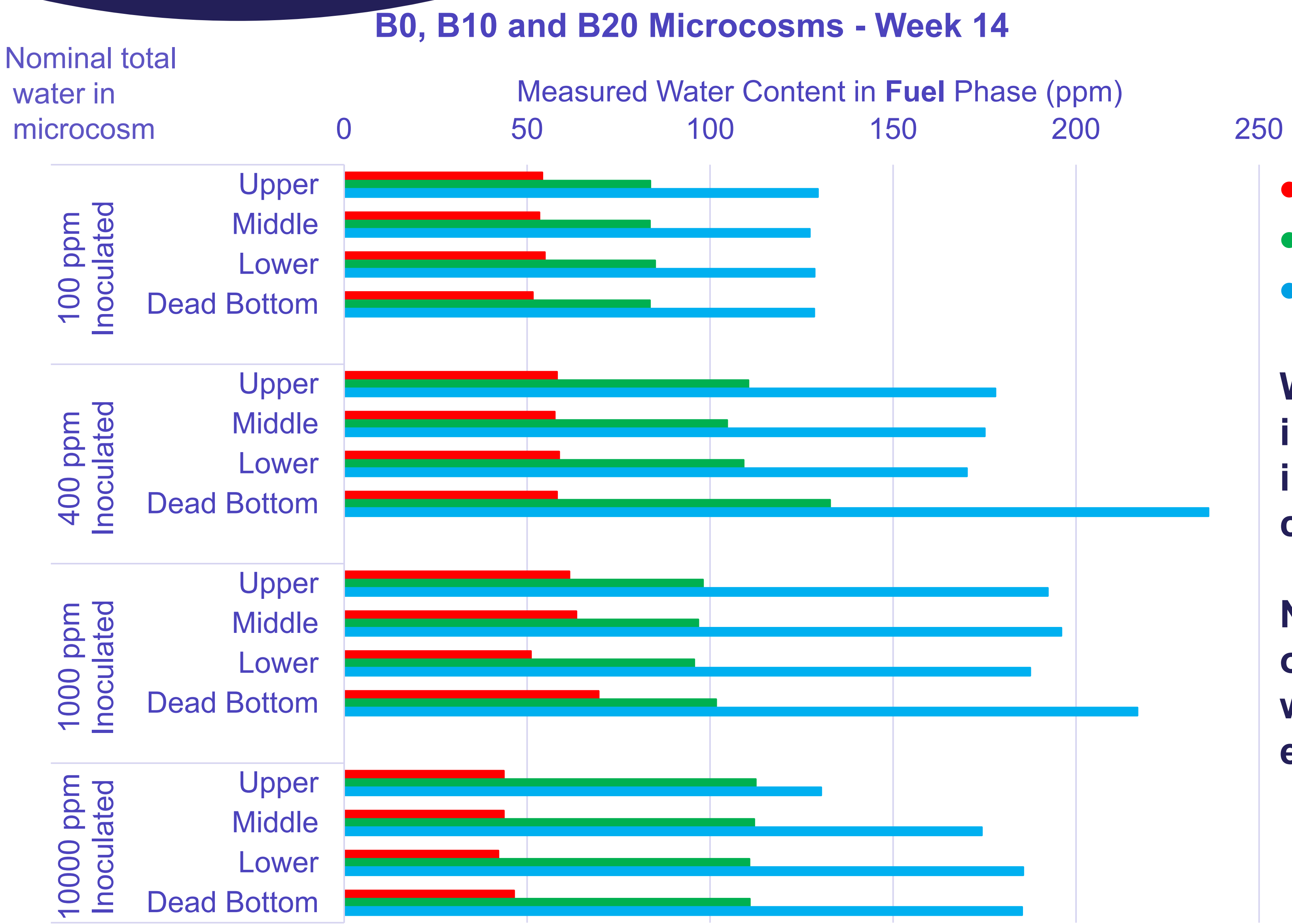
- TVC of aqueous phase in microcosms set up to contain 1000 and 10000 ppm total water.



● Bacteria ● Yeasts ● Moulds

- Shift from bacterial growth to fungal growth with increasing FAME content

Influence of FAME on distribution of water in diesel

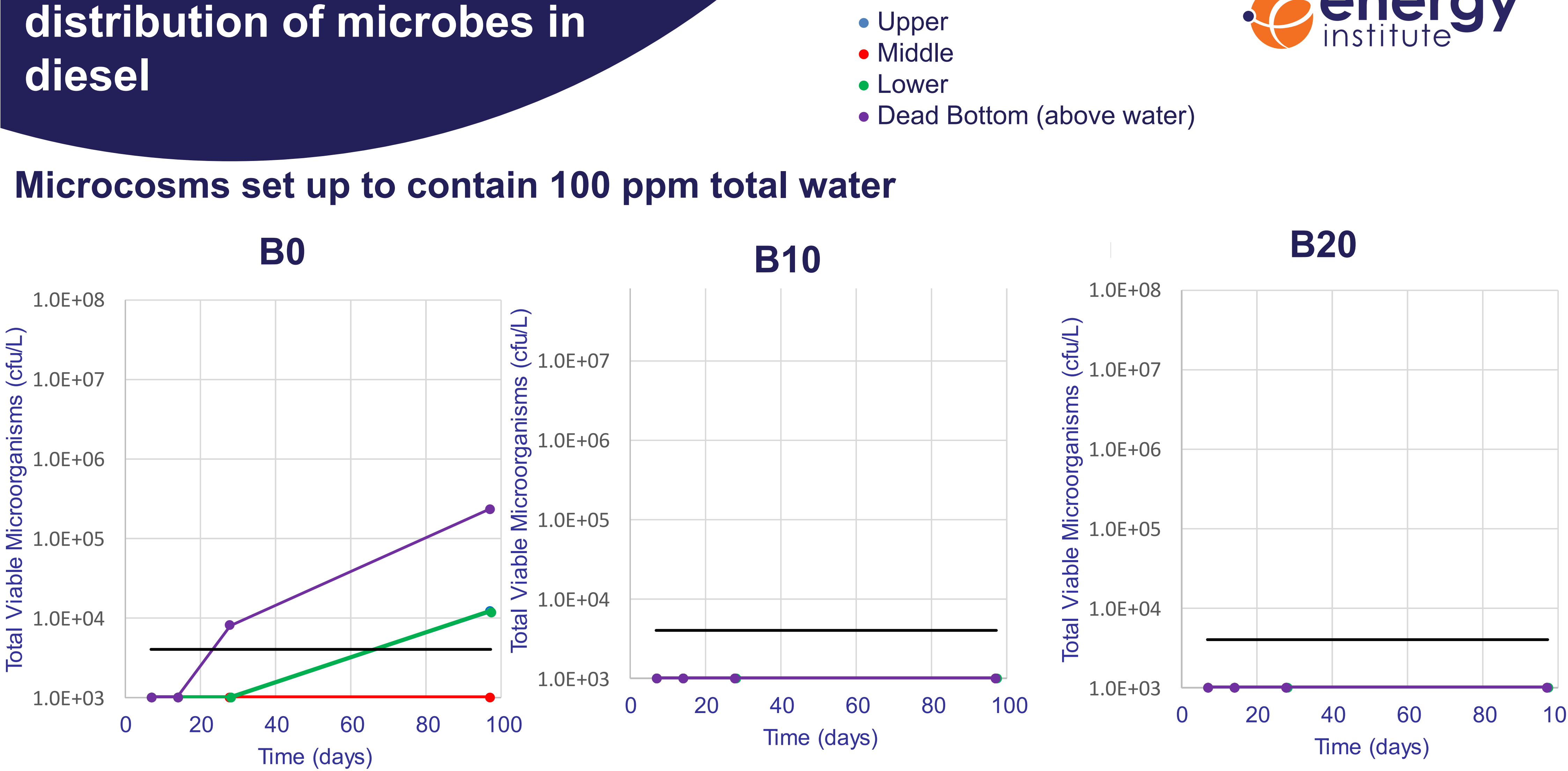


Water content in fuel increased with increasing FAME concentration.

Note more stratification of water in B10 and B20 when total water added exceeded 400 ppm.

Influence of FAME on distribution of microbes in diesel

Microcosms set up to contain 100 ppm total water



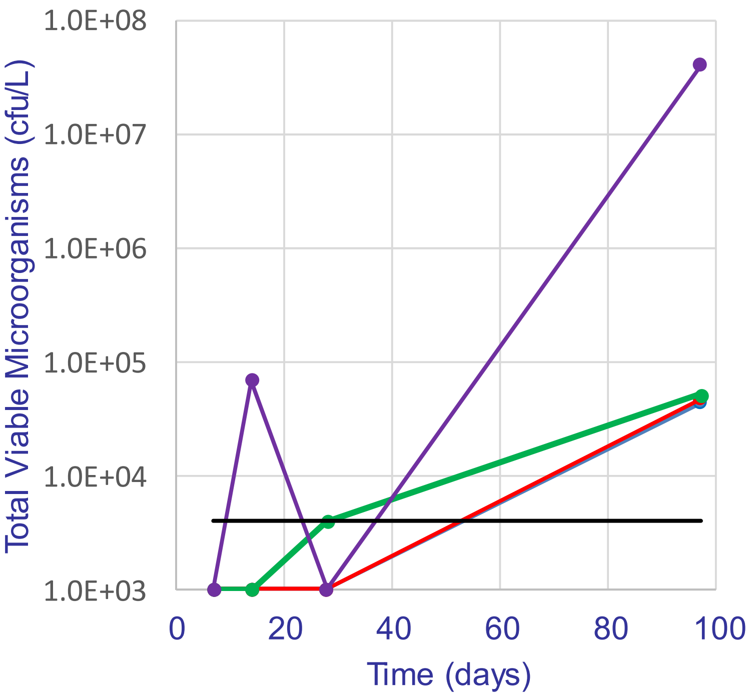
- **Microbes detected at dead bottom, lower, upper fuel layers in B0 microcosm.**
- **No microbes detected in fuel layers in microcosms containing B10 or B20 (even though there were aggregates of viable microbes in the microcosm bottom).**

Influence of FAME on distribution of microbes in diesel

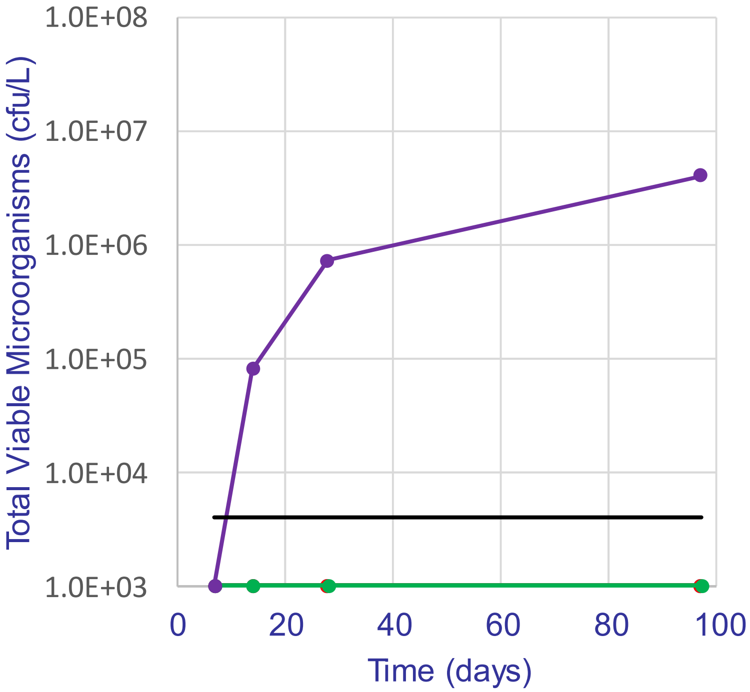
- Upper
- Middle
- Lower
- Dead Bottom (above water)

Microcosms set up to contain 400 ppm total water

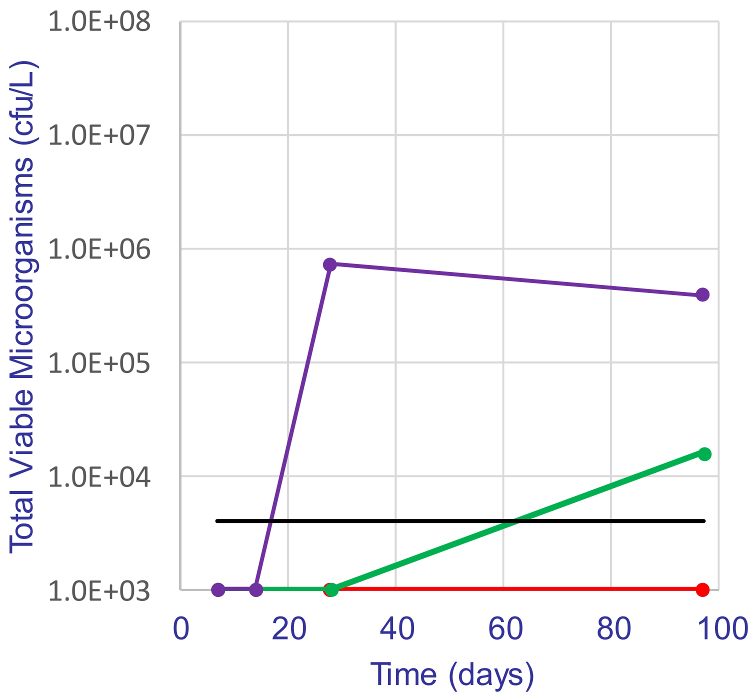
B0



B10



B20

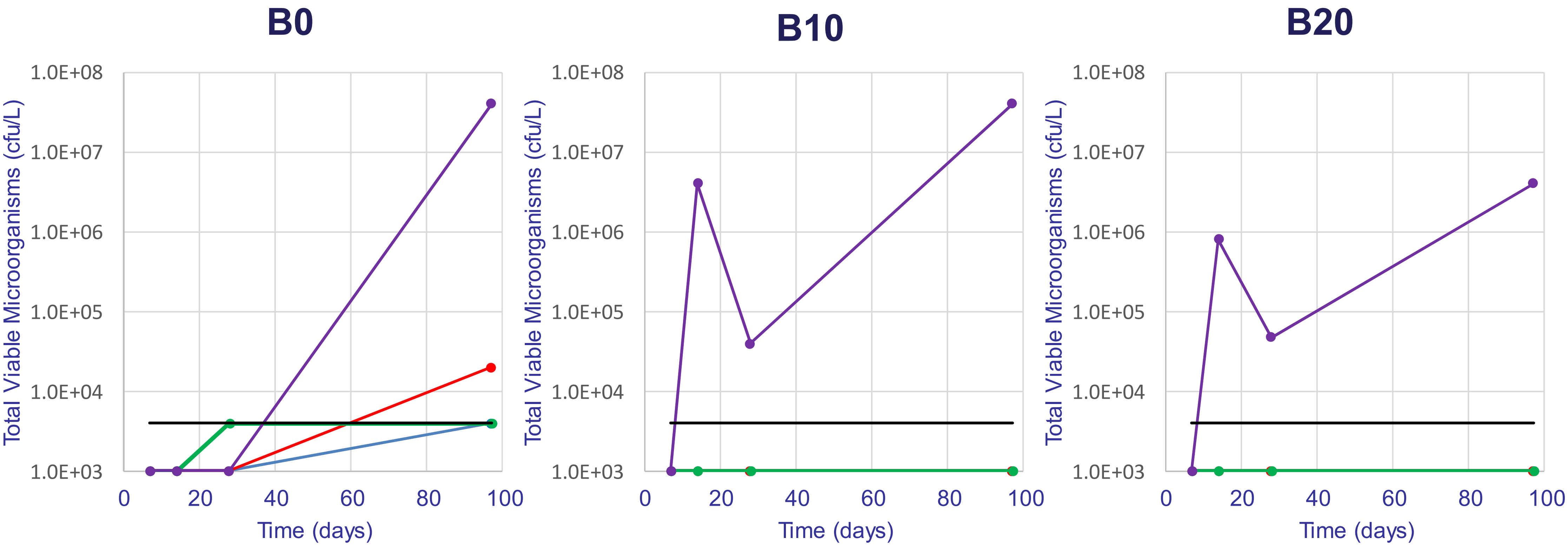


- **Microbes present in fuel layers above the dead bottom position in B0 microcosm.**
- **Few if any viable microorganisms in upper, middle & lower fuel layers of B10 and B20.**

Influence of FAME on distribution of microbes in diesel

- Upper
- Middle
- Lower
- Dead Bottom (above water)

Microcosms set up to contain 1000 ppm total water

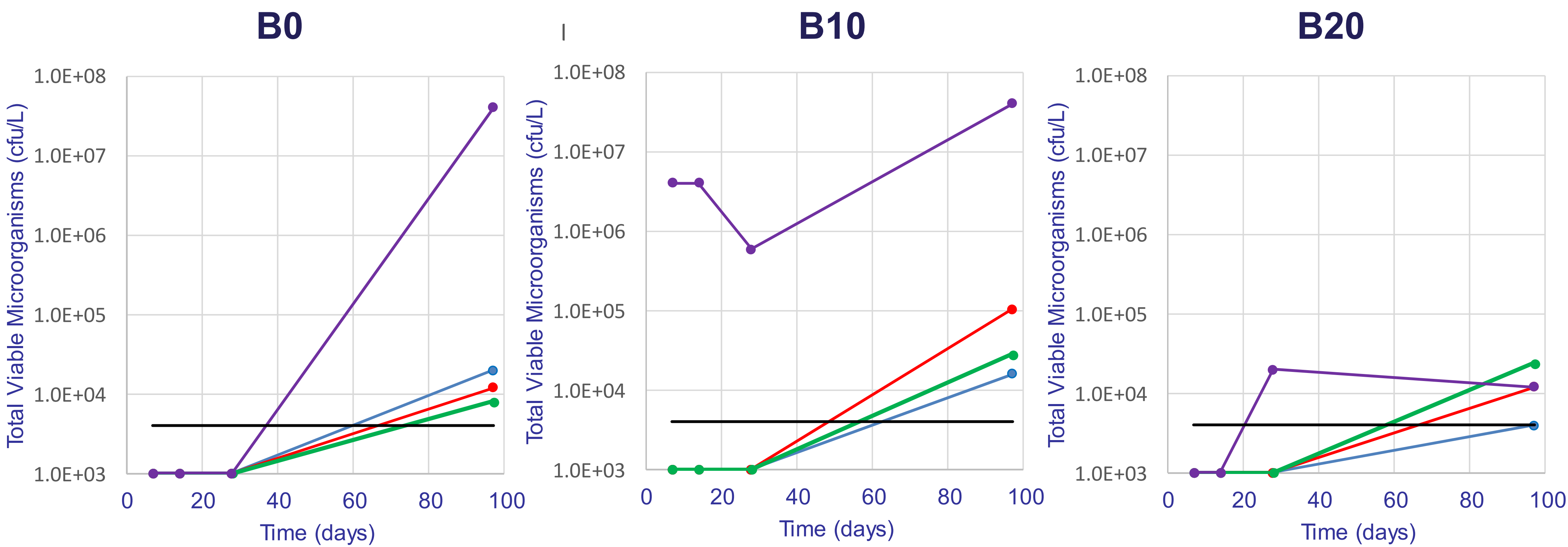


- **Microbes present in fuel layers above the dead bottom position in B0 microcosm.**
- **No viable microorganisms in upper, middle & lower fuel layers of B10 and B20.**

Influence of FAME on distribution of microbes in diesel

- Upper
- Middle
- Lower
- Dead Bottom (above water)

Microcosms set up to contain 10000 ppm total water



- **Microbes present in fuel layers above the dead bottom position in B0, B10 and B20 microcosms.**
- **Similar distribution of microbes for all FAME blends.**

Other findings

- **TAN and FBT of fuel;**
  - Increased with increasing FAME concentration.
  - Cannot confirm influence of microbial content given samples were from the middle layer and microbial contamination was restricted to microcosm bottoms.

Other findings

- **Un-inoculated microcosms;**
  - Water content increased with increasing FAME concentration as for inoculated microcosms.
  - Light microbial contamination detected in base fuel before the study commenced.
    - Microbial growth developed in the microcosms set up to contain 10000 ppm total water.

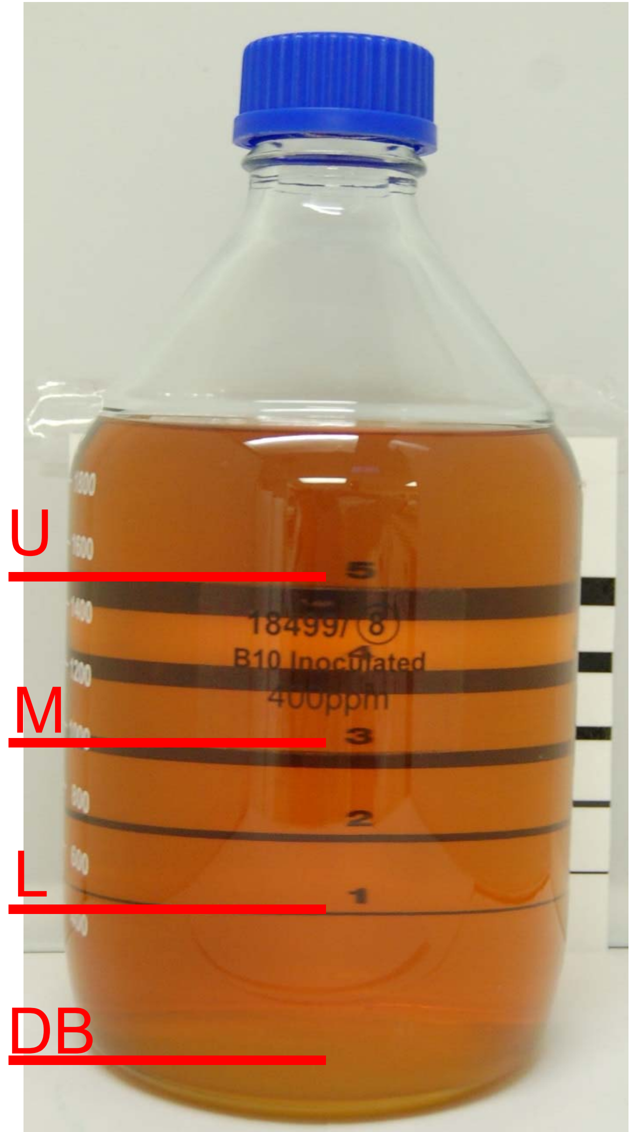
Part 1 Summary

- **Influence of FAME on microbes and water.**
  - Overall microbial growth increased with increasing FAME concentration.
  - Shift from bacterial growth to fungal growth with increasing FAME content.
  - Water content in diesel increased with increasing FAME concentration.
  - However, microbial contamination in diesel phase (upper, middle, lower) did not generally correlate with the water content detected at each level.
  - Even though microcosms were agitated weekly, the vast majority of microbial contamination remained in the bottom, even when relatively high water contents were detected in fuel.
  - Irrespective of FAME concentration when nominal total water in microcosm was below 100 ppm very little microbial growth was observed.

PART 2 Overview

Part 2: Influence of settling time on the distribution of water and microbes.

- Conducted on 2 microcosms from PART 1;
  - B10 and B20 microcosms set up to contain 400 ppm total water.
- Microcosms vigorously shaken.
- Samples taken at 4 depths immediately after shaking and then after 1, 2, 6, 12, 24 and 48 h.
  - Total Viable Count (TVC) by IP 613/14 (ASTM D7978-14).
  - Water content by Karl Fischer IP 438/01.



PART 2 Distribution of viable microbes (IP 613) with settling time

B10 with 400 ppm total water

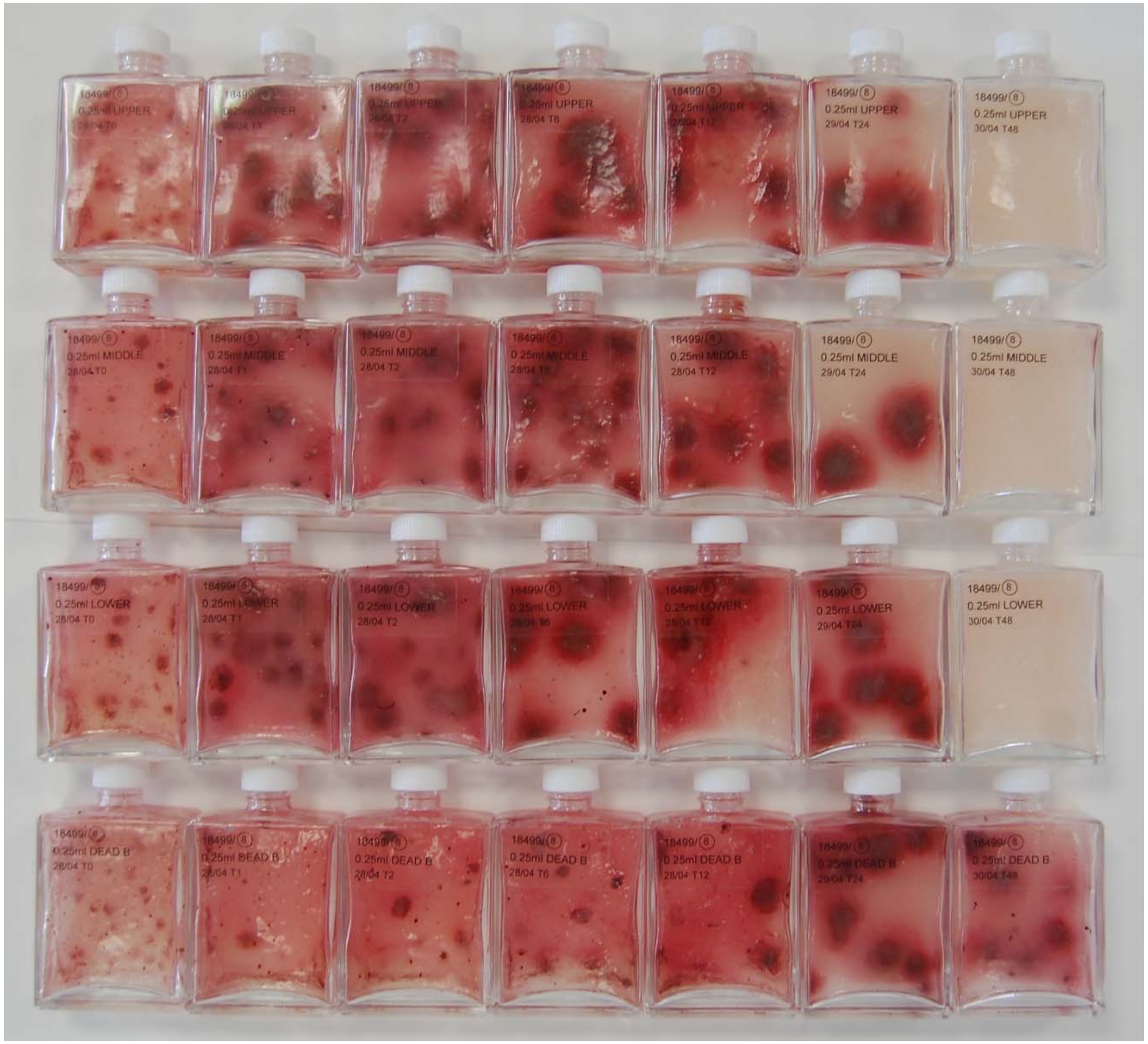
0 1 2 6 12 24 48 (h)

Upper

Middle

Lower

Dead Bottom



20 cm

PART 2 Distribution of viable microbes (IP 613) with settling time

B20 with 400 ppm total water

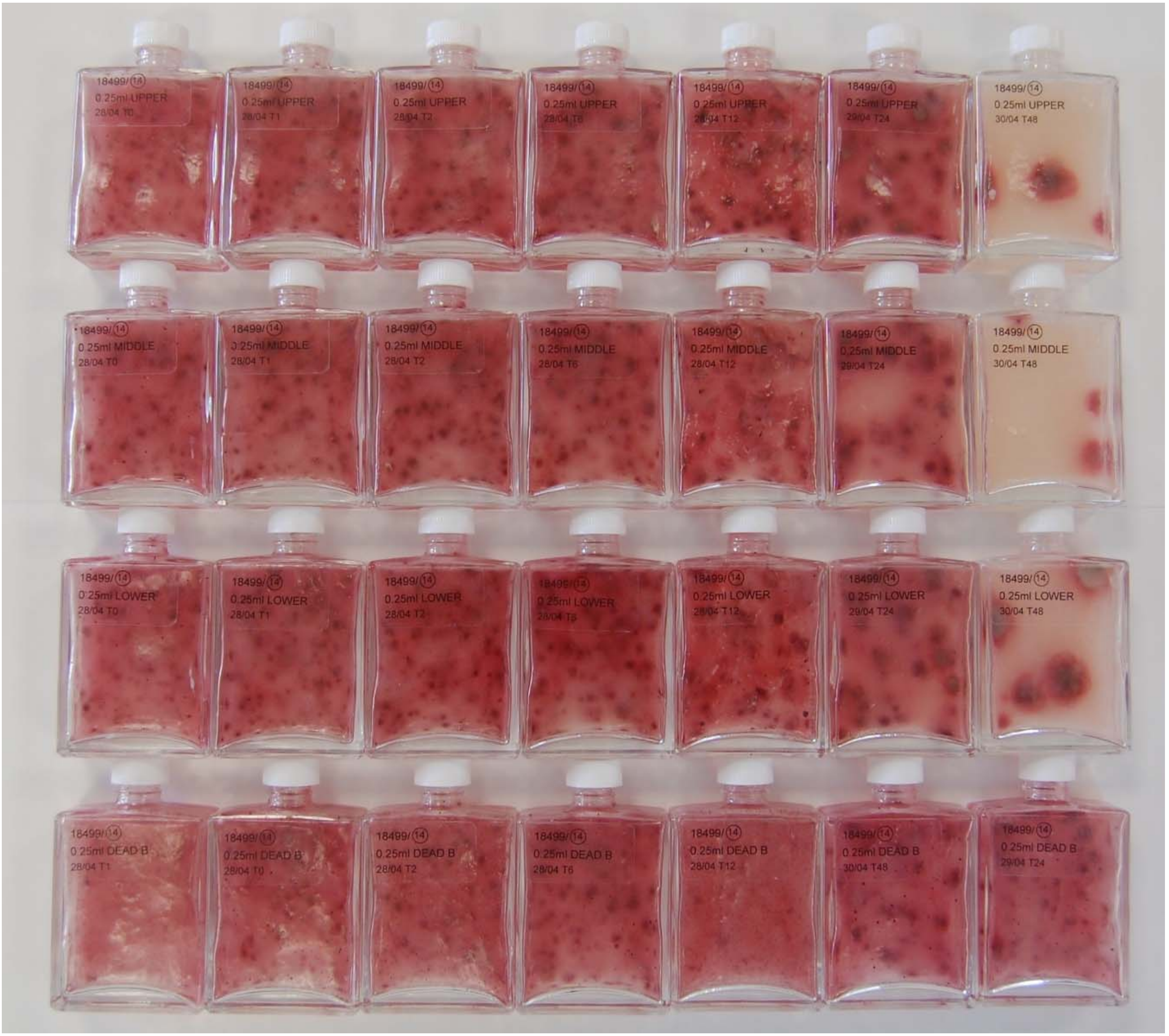
0	1	2	6	12	24	48	(h)

Upper

Middle

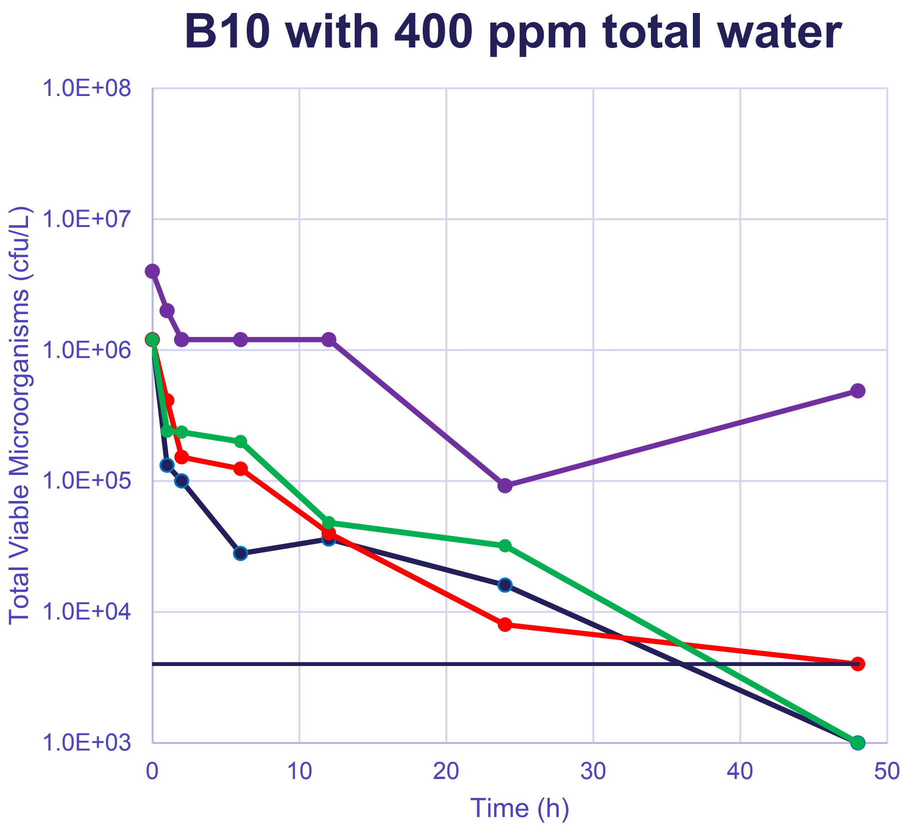
Lower

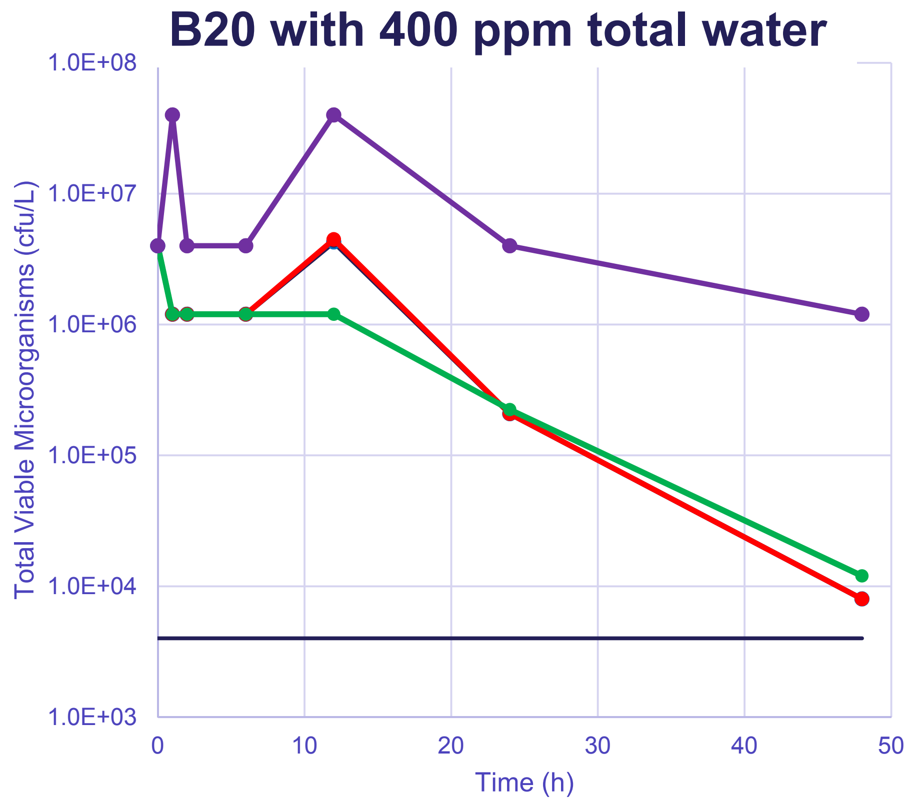
Dead Bottom



20 cm

PART 2 Distribution of viable microbes with settling time





- Layers above dead bottom relatively free of microbiological contamination after 48 h
- Microbes still detected in layers above dead bottom after 48 h
- Water content data was confusing! Lower water contents detected in fuel 48 h after shaking than were detected in Part 1 study where microcosms were only agitated gently on weekly basis.

Part 2 – Summary

- **Settling rate quicker for B10 compared to B20**
- **B10 settling rate;**
  - 90% of CFU; 7 – 14 cm/h
  - All CFU; 0.3 – 0.6 cm/h
- **B20 settling rate;**
  - 90% of CFU; 0.6 – 1.2 cm/h
  - All CFU; <0.3 cm/h
- **Commonly used industry guidance is to allow a settling time of 3 h per meter of product height after product receipts.**
  - For B10 this recommended settling time is likely to be just adequate
  - For B20 this recommended settling time is likely to be inadequate

Conclusions

- Regularly drain free water AND “wet” bottom fuel from tank bottoms to limit the potential for microbial growth and biomass accumulation.
- Longer settling time may be needed after receipts into microbially contaminated B20 tanks.

Acknowledgements

- **Energy Institute**
  - Microbiology Committee, in particular;
  - Ramya Vankataraman (Exxon Mobil)
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