



DGMK Field Study 2007/09

Diesel Fuels with FAME and Microbs

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DGMK Organisation

- DGMK - 'technical research association' of the German branches of mineral oil companies
- DGMK working groups: Fuels, Heating Oil, Supply&Distribution, Refineries
- Objective of the 'Fuels working group': Create and run technical investigation programmes about German fuels quality aspects
- Majority of current DGMK fuels projects are about biofuels, several about FAMES in diesel fuels (OEM concerns)
- DGMK – a mineral oil company association. However, for several biofuels projects DGMK has invited OEMs, FAME suppliers, additive companies for joined work

Background for the DGMK project about microbes

- Scattered problems of filter clogging at diesel dispensers and deposits in depot tanks occurred since intro of B5 in Germany from 2005 on
- During winter 2005/06 about 200 retail dispensers were affected, not such problems before B5 intro
- Problems have reduced now, but not completely gone

First DGMK spot checks about filter blocking found

- No relation to a specific region in Germany
- No impact of different technical configurations of dispensers, filters
- Some B5 terminal tanks were microbial infected but not all supplied retail stations

Appearance of deposits in a terminal tank



Slimy deposits at wall and bottom of a B5 terminal tank



Hole corrosion beneath slime deposits

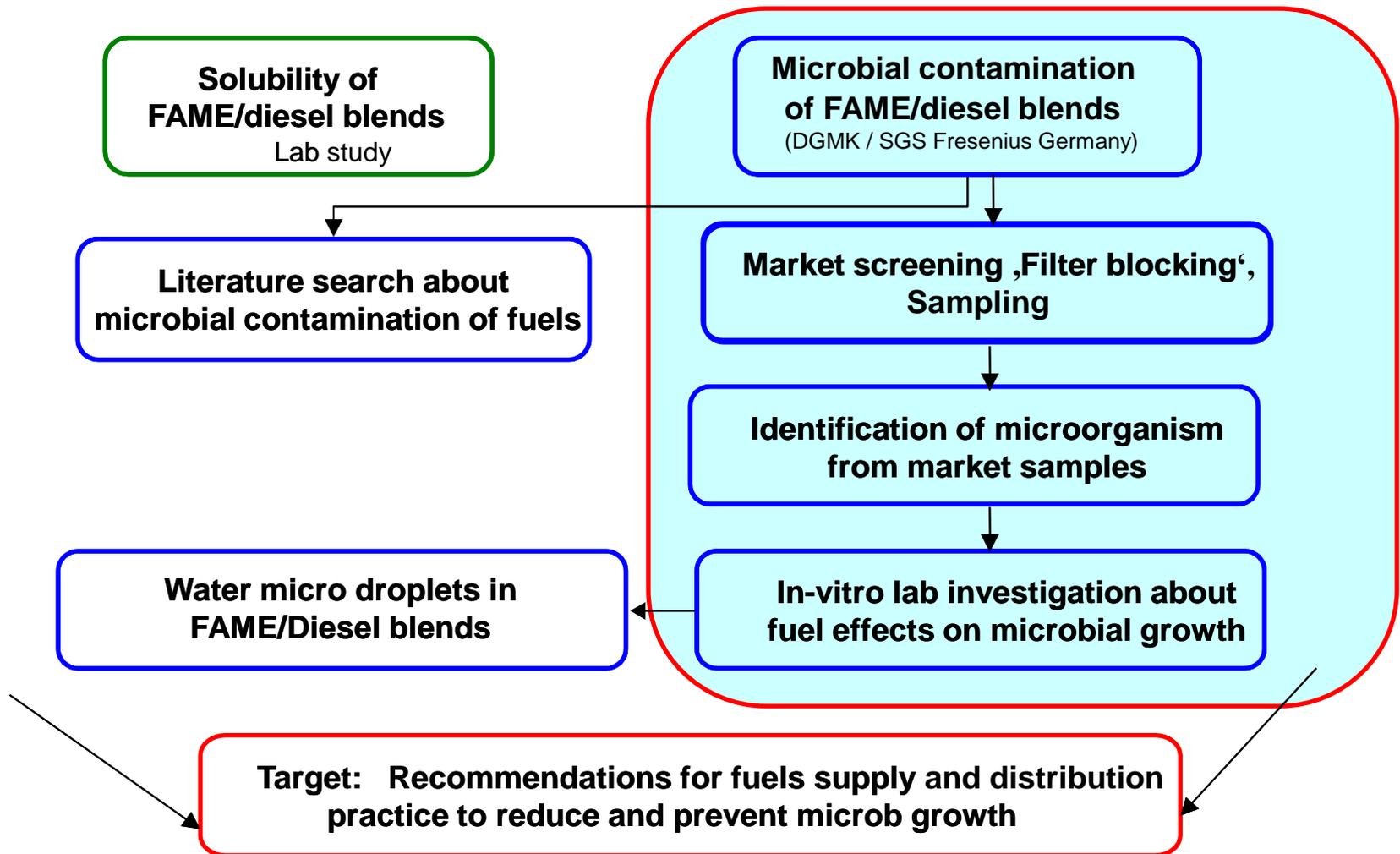
Appearance of blocked dispenser filters



- Majority of blockings appeared as typical dark slimy deposits
- Initial lab tests of the deposits showed **always high microbial contamination**

- Only a few blockings obtained as light fine deposits
- Analysis: FAME associated contaminants as sterolglucosides and others

DGMK approach to investigate the filter blocking phenomena



Market screening along the B5 supply chain Germany

- 150 fuel and deposit samples investigated with microbiological methods
- Results:

Supply chain	terminal B5 tanks		retail station B5			
↓ Sampling location	bottom phase	fuel from middle	tank bottom phase	fuel from tank middle	deposits at dispenser filters	fuel out of dispensers
↓ Contaminated samples out of total at location >10 ¹ cfu/ml	25%	38%	41%	62%	95%	52%
↓ Range of contamination (bacteria, fungi, mould) cfu/ml	10 ⁵ - 10 ⁷	10 ¹ - 10 ²	10 ¹ - 10 ⁵	10 ¹ - 10 ⁶	slight to heavy biomass deposits	10 ¹ - 10 ⁶

Visual details about microbial contamination along the supply chain

***terminal tank
bottom sediments***



terminal tank fuel samples

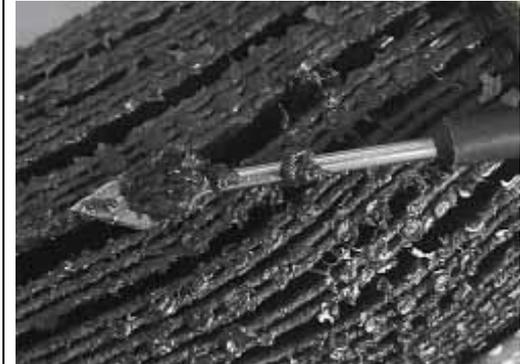


fungi hyphae and micro water droplets in the fuel phase, ca. 800x

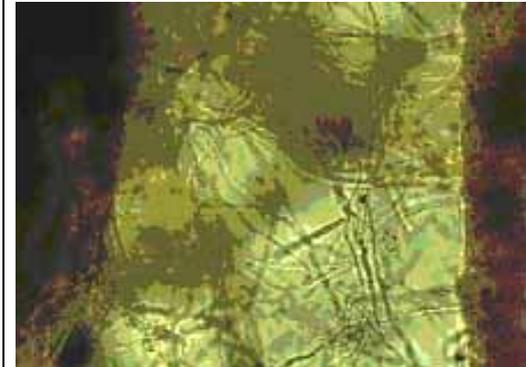


fungi hyphae in the diesel fuel phase (yellow), extend into water phase (dark), ca. 800x

deposit material from dispenser filter



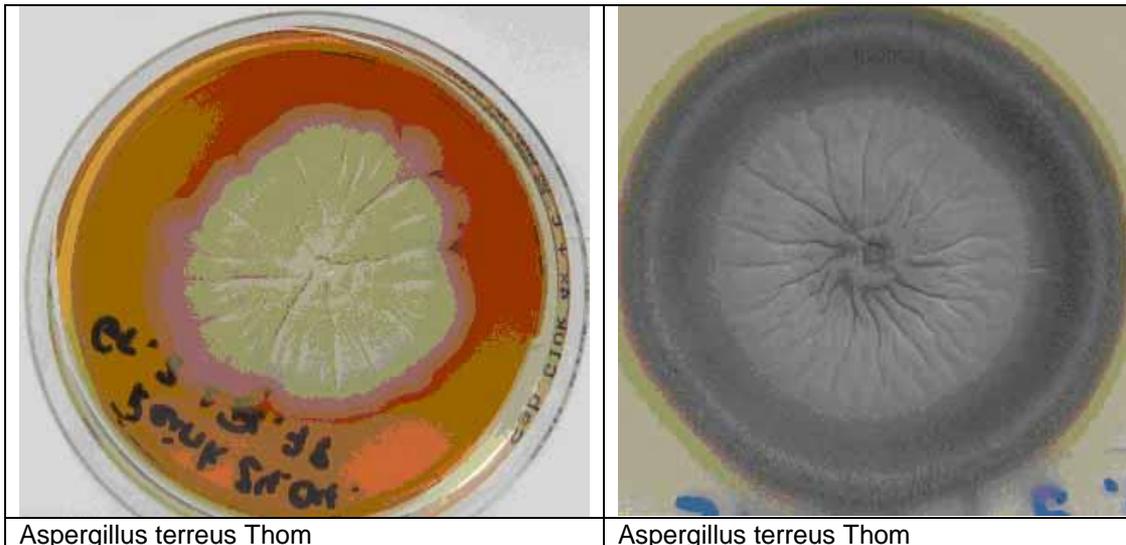
microbial biomass deposits at dispenser filter paper



fungi hyphae between cellulosic dispenser filter material, ca. 800x

Identification of microorganism from market samples

- **Both** bacteria **and** fungi identified to comparable numbers
- **But:** fungi mycelia and hyphe meshworks turned out as main reasons for filter blocking in the field
- All identified fungi classified 'not or very unlikely to cause harm for humans' TRBA 460
- Examples for isolated and cultivated fungi species:



In-Vitro lab investigations about fuel effects on microbial growth

- Do certain fuel parameters promote microbial contamination and growth?
- Fuels matrix: 1 base diesel, 4 FAMEs (from 2 RMEs) with variations in P, water content, lecithin → 4 B5 fuels, w/wo free water phase
- Preparation of 2 microbial inoculums:
 1. **Defined fungi mix** (10 numerous fungi, *isolated and cultivated* from field samples)
 2. **Complex biomass** (microb mix *extracted* from field samples)

Inoculums were **dry-frozen** to prevent any uncontrolled water impact

Injected with Defined fungi inoculum	
no free H ₂ O phase	free H ₂ O phase added
Base diesel	Base diesel
B5 -1 (FAME1)	B5 -1 (FAME1)
B5 -2 (FAME2)	B5 -2 (FAME2)
B5 -3 (FAME3)	B5 -3 (FAME3)
B5-4 (FAME3)	B5-4 (FAME3)
FAME 1	FAME 1

Injected with Complex biomass inoculum	
no free H ₂ O phase	free H ₂ O phase added
Base diesel	Base diesel
B5 -1 (FAME1)	B5 -1 (FAME1)
B5 -2 (FAME2)	B5 -2 (FAME2)
B5 -3 (FAME3)	B5 -3 (FAME3)
B5-4 (FAME3)	B5-4 (FAME3)
FAME 1	FAME 1

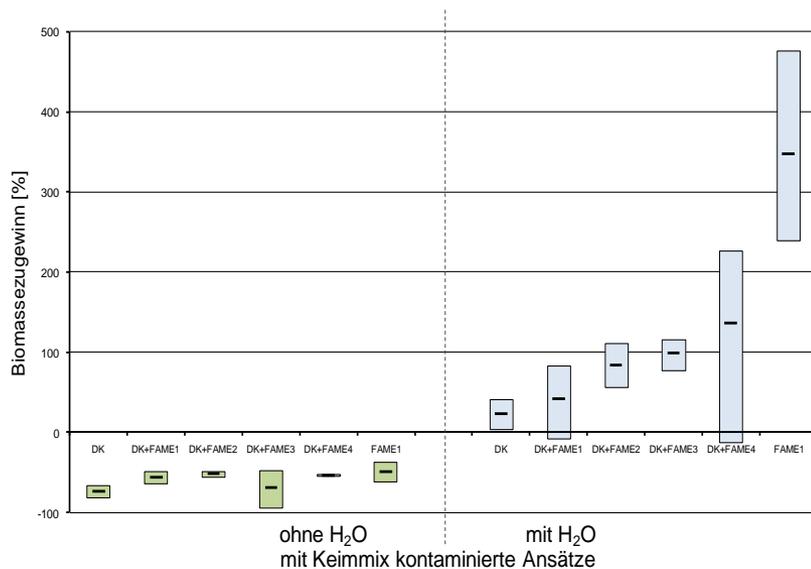
- Microbial measurements: Biomass growth, ATP, DNA, RNA, proteins

In-vitro lab investigations about fuel effects on microbial growth

Results (Biomass growth exemplary)

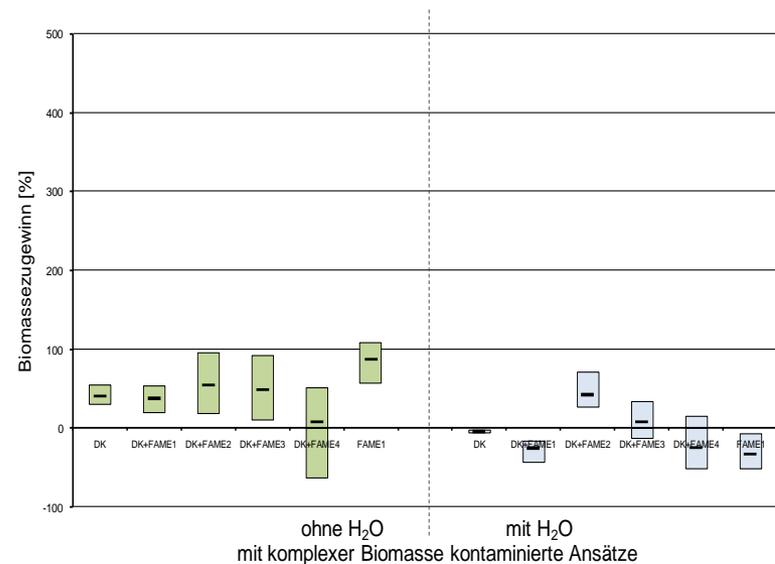
Defined fungi inoculum

- free water phase → clear tendency to increased biomass growth for all fuels
- no correlation between microbial growth and FAME composition or contaminants (e.g. FAME 2, 4: higher P)
- Pure FAME with free water phase added showed rel. strongest microb growth



Complex biomass inoculum

- results different to defined inoculum
- free water phase → no biomass growth compared to defined inoculum
- even tendency to reduced growth compared to without water phase
- conflicting with expectations: complex biomass seen as near 'real life' compared defined inoculum



Summary

- Market survey Germany:
 - Microbial contamination found as main reason for retail filter blocking issue with B5 fuels
- In-vitro lab programme:
 - It is known from practice that a free water phase in diesel tanks can stimulate microbial growth
 - However, only the fuel sample set which was doped with the defined funghi inoculum could reproduce this effect
 - These findings did not match our expectations: just the complex biomass inoculum seen as 'natural' microb mix should represent 'real world' microbial contamination better than a lab prepared defined fungi inoculum
 - This contrasting result might be a caused by competition of bacteria vs. fungi metabolism in the complex biomass inoculum, impact of microb growth inhibition components or others
- The investigation about 'real' existence of micro water droplets in FAME containing diesel fuels and a potential stimulation effect on microbial growth was not successful

Recommendations from the DGMK project for fuels practice in order to minimize risks of microbial growth

- For FAME purchasing: not fully utilise EN 14214 water spec of max. 500 ppm, regularly analysis of FAME batches for water content
- Minimise the occurrence of free water phases in supply and distribution systems by consistent best practice housekeeping
- No *sustainable* improvement could be achieved with the biocides so far used in the tanks at service stations
- Tank cleaning at service stations in combination with disinfection of the system with hydrogen peroxide provided better prevention of filter clogging than the biocide treatment

Thank you !