

MWF surfactant:
Biocide booster,
or not biocide booster,
that is the question

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Definition

Surfactants (blend of surface-active agent*)

- chemical compounds that decrease the surface tension between

- two liquids,
- liquid and a gas
- liquid and a solid.

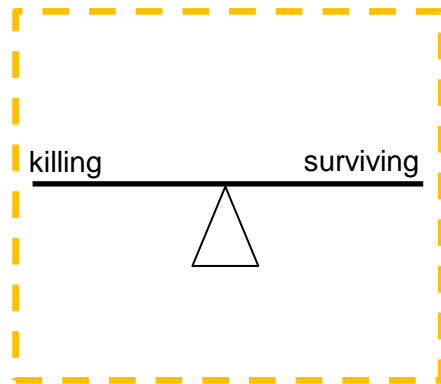
Surfactants:

- detergents and cleaning agents
- emulsifiers
- wetting agents
- foaming agents
- antistatic additives
- dispersants

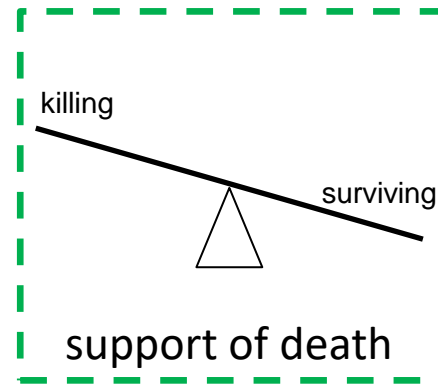
How additives modulate biocides

Three general options

neutral additives
„neutral“ to susceptibility

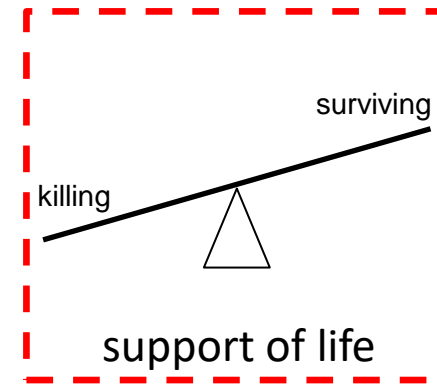


synergistic additives
„booster“ of susceptibility



good for MWF

„anagonistic“ additives
„inhibitors“ of susceptibility



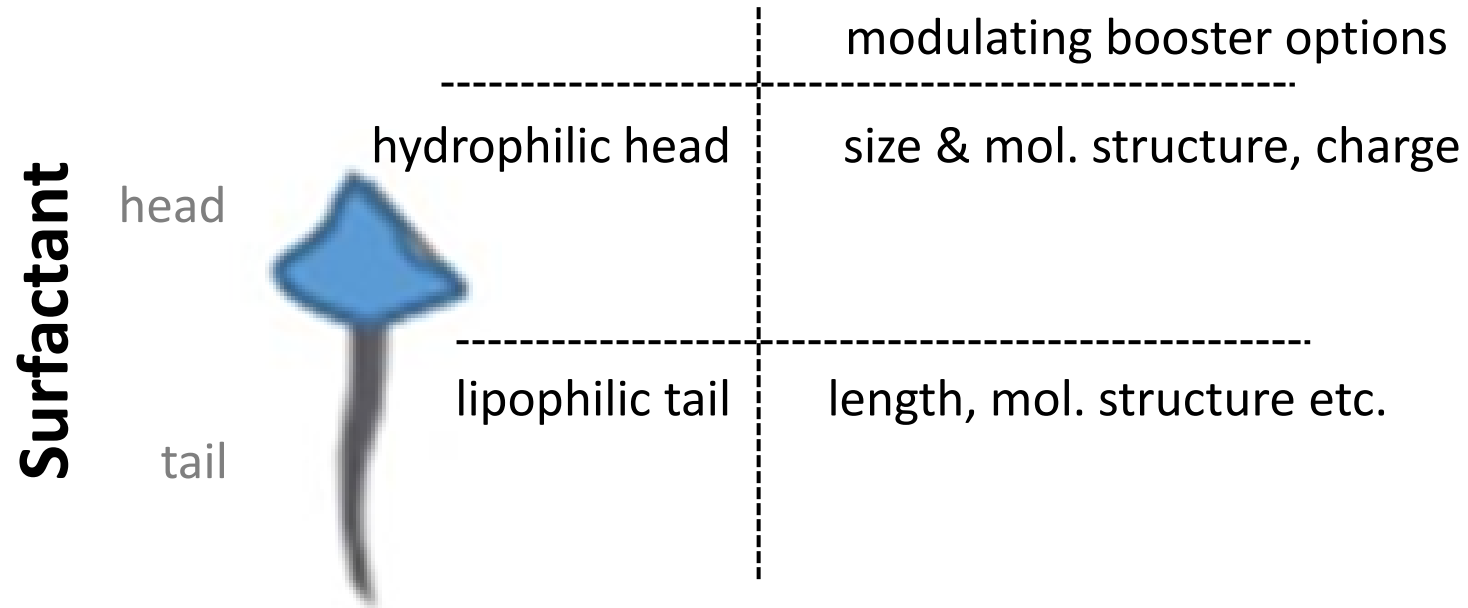
bad for MWF

e.g. compounds easy to metabolize

1st Take Home Message

Each additive can change positively or negatively the effect of biocides in a MWF

Molecular structure of surfactants modulate booster options



Source: chemical-based
microbial (low & large molecular weight)
natural-based (plant- & oleo-based)

Surfactants can be both booster or inhibitors of biocide susceptibility

- ❖ **Microbial life** occurs at the **oil-water interface**. Hence **surfactants increase the surface** of oil droplets in the water and support nutrient supply of the microbes.
- ❖ Depending on the concentration and kind of **surfactant's lipophilic tail**, the bacterial cell **membrane can be damaged**.
- ❖ Depending on the concentration and kind of **surfactant's hydrophilic head**, the bacterial **membrane proteins can be damaged**.

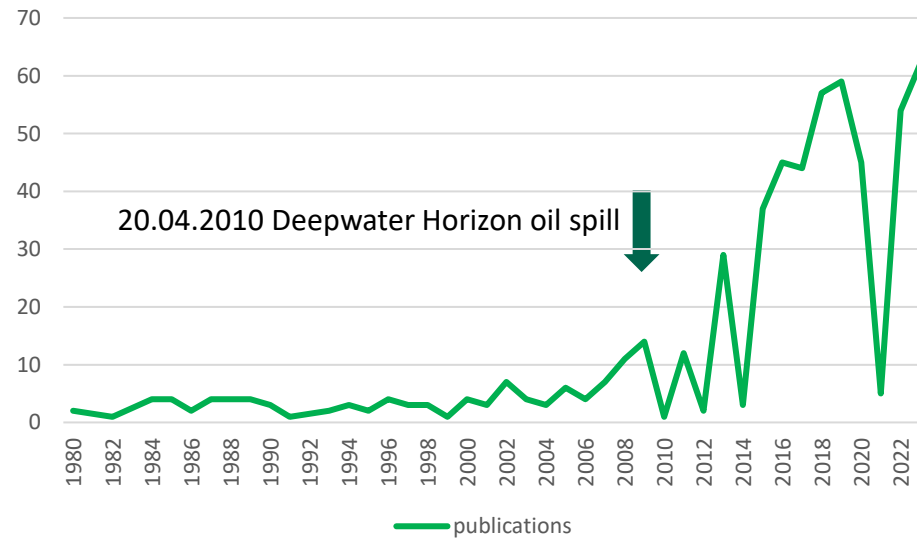
**MWF surfactant:
Biocide booster,
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Surfactants as biocide booster?

Starting signal for booster R&D: Oil spill 2010

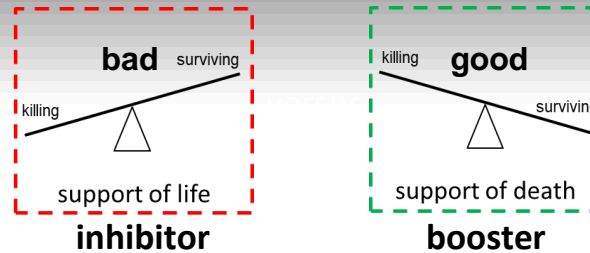
Increase of research on surfactant and microorganisms

NCBI Search query (12/2023): dispersants, oil, bacteria, environment



Surfactants as biocide booster?

a closer look into the literature



Corexit 9500 (dispersant of BP; untested and off-label use after Deepwater Horizon oil spill)

Taxa of oil-degrading bacteria (e.g., *Oleispira*, *Polaribacter*, and *Colwellia*) **increased** in relative abundance **in response to both oil and Corexit 9500**.

(McFarlin et al. *Frontiers in Microbiology* 2018; DOI: 10.3389/fmicb.2018.01788)



Tergitol NP-10 and resulted in an overall **increase** in the **growth** with phenanthrene of *Pseudomonas stutzeri* (Grimberg et al. *Appl Environ Microbiol* 1996;62(7):2387-92.doi: 10.1128/aem.62.7.2387-2392.1996.)



Tween 80 vs Triton X-100: *Sphingomonas* strains — the rate of fluoranthene **mineralization** was **almost doubled**. By contrast, the same **surfactant inhibited the rate** of fluoranthene **mineralization** by two strains of *Mycobacterium*. **Triton X-100 inhibited** fluoranthene mineralization by **all strains**

(Willumsen et al. *Applied Microbiology and Biotechnology* 1998;50:475–483)



Bruheim P et al. Bacterial degradation of emulsified crude oil and the effect of **various surfactants**. *Can J Microbiol* 1997, 43:17-22.

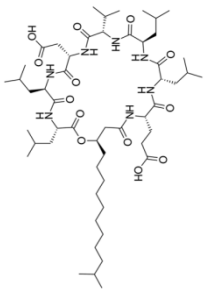
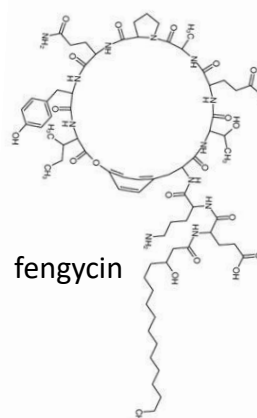
etc. (more than 300 publication about dispersants, oil, bacteria, environment – a treasure chest for biocides MWF-booster)

2nd Take Home Message

The worse a surfactant supports the growth of bacteria and fungi, the more effective the biocide works in a MWF

Examples of microbial biosurfactants

Table: Salome Dini et al. Molecules 2024, 29, 2544

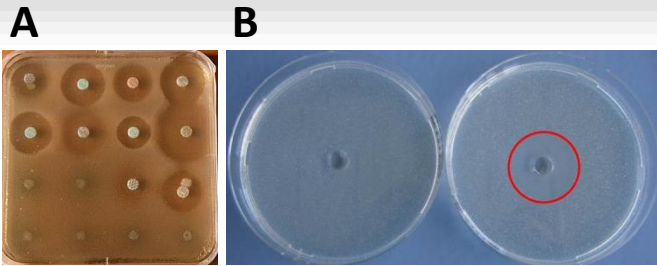
Functional Properties	Biosurfactant Types	Microorganism Isolates	Functional Properties	Biosurfactant Types	Microorganism Isolates
 surfactin Antibacterial activities	Lipopeptides (<u>surfactin</u> , iturin and <u>fengycin</u>)	<i>Bacillus amyloliquefaciens</i> C-1	Antifungal activities	Lipopeptide (<u>fengycin</u> and iturin)	<i>Bacillus amyloliquefaciens</i>
	Lipopeptides (Brevilaterin B)	<i>Brevibacillus laterosporus</i> S62-9		Lipopeptides (<u>mycosubtilin</u> and surfactin)	<i>Bacillus</i> sp.
	Lipopeptides (surfactin)	<i>Bacillus velezensis</i> SK		Lipopeptides (<u>fengycin</u>)	<i>Bacillus amyloliquefaciens</i> PPL
 fengycin Antibiofilm activities	Rhamnolipids	NR	Antiviral activities	Rhamnolipids	<i>Pseudomonas gessardii</i> M15
	Glycolipoprotein	<i>Oceanobacillus</i> sp.	Glycolipids	<i>Azadirachta indica</i>	
	Glycolipoprotein	<i>Lactobacillus plantarum</i> 60 FHE	Glycolipid	<i>Bacillus pumilus</i> SG	
	Sophorolipid	<i>Candida</i> spp.	Lipopeptides (<u>bacillomycin</u> D)	<i>Lactobacillus rhamnosus</i>	
				Lipopeptides (<u>bacillomycin</u> D)	<i>Bacillus subtilis</i> TR4711
			Glycolipoprotein	<i>Acinetobacter</i> M6	
			Lipopeptides	<i>Halomonas venusta</i> PHKT	

— already declared as biocide

- - - - the declaration depends on the usage

Methods to test biocide booster in MWF

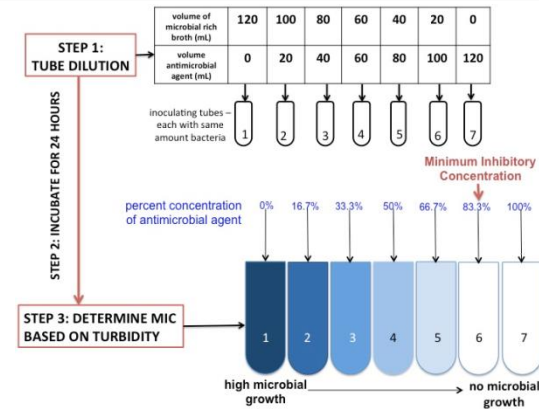
disc diffusion test (not frequently used)



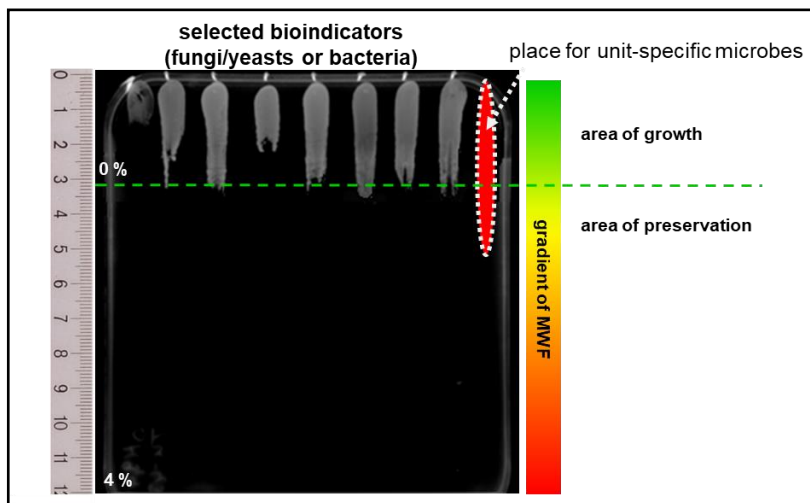
temp. depending on the strain used

broth dilution assay (not frequently used)

temp. depending on the strain used

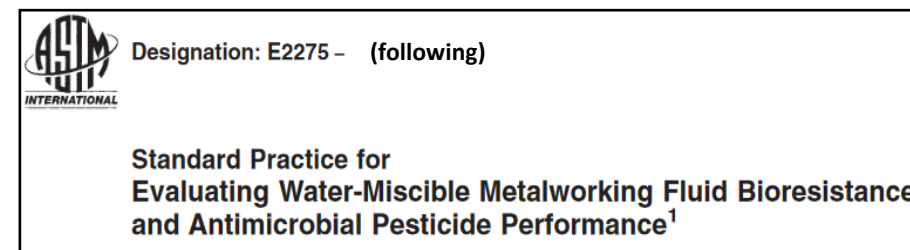


MWF-biomonitoring (quick screening)



temp. 30°C

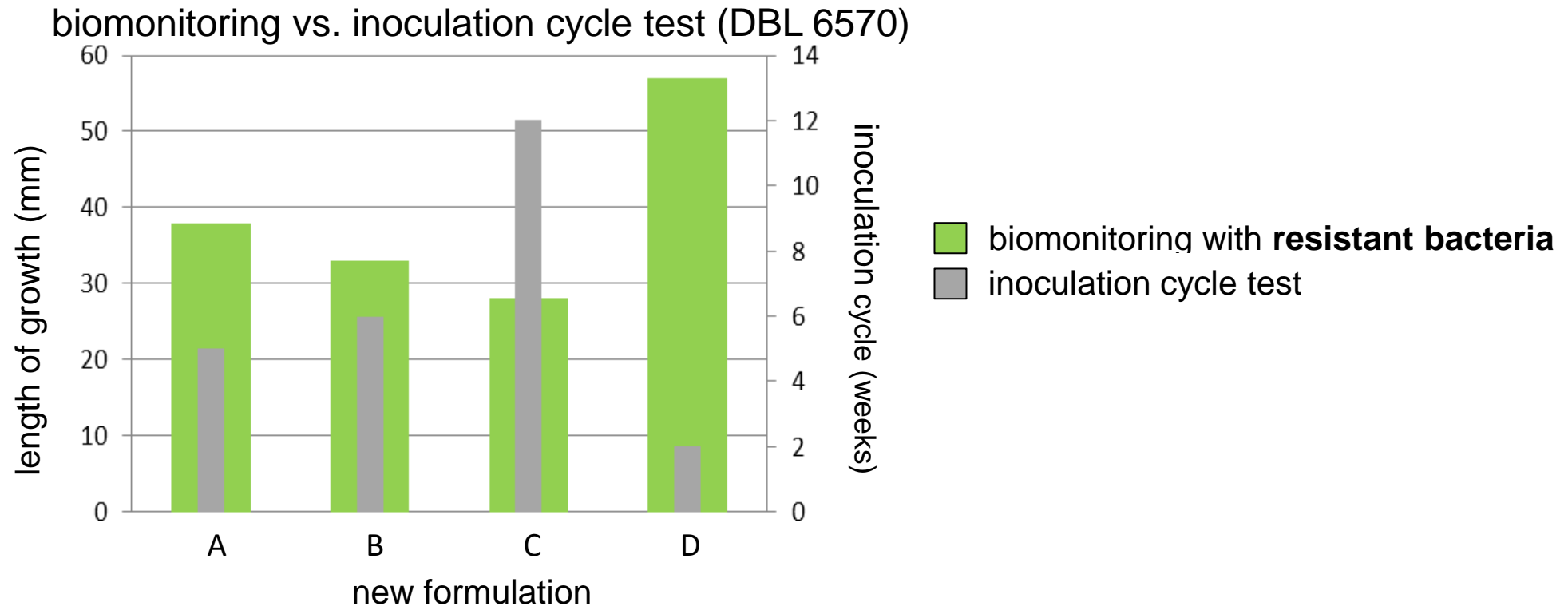
inoculation cycle test (long lasting – close to application)



analogous DBL 6570 (Mercedes-Benz)

both temp. 25°C

Comparability of biomonitoring and inoculation cycle test



3rd Take Home Message

- ❖ Due to global warming antimicrobial tests should be done at higher temperature (e.g. $\geq 30^{\circ}\text{C}$)
- ❖ Microbes from used emulsions in combination with standard microorganisms should always be tested to hit the goals:
 - broad view onto all MWF-microbiomes
 - comparability between MWFs

Summary and concluding remarks

- ❖ In the last decade, science has described numerous surfactants that could function as part of antimicrobial strategy in MWFs.
- ❖ These booster candidates should be tested individually and in combination with other additives.
- ❖ By appropriate testing procedures, we will develop new antimicrobial strategies to overcome the challenges of:
 - the global climate change
 - the tightening of regulatory conditions
 - the increasing development of microbial unsusceptibilities

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