Desinfektion/ desinfektionsmidler: Hvordan og med hvilke midler rammer vi mikroorganismerne?

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Agenda

Desinfektion/desinfektionsmidler

- Fysiske
- Kemiske

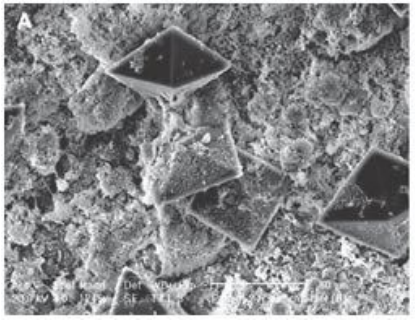
Hvad påvirker effektiviteten?

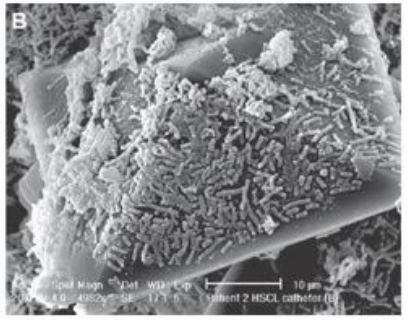
- Mikroorganismer
- Overflader
- Matrix incl. biofilm

Øget smittepres kan nødvendiggøre desinfektion

Mere generelt

- Når der er mange samlet og risikoen for smittespredning er stor
- Når der er immunologisk sårbare til stede
- Men også når mikrobiel vækst skaber tekniske problemer





Undgå smitte i produktion og pleje (men også forøge holdbarhed af fødevarer)

Man kan definere desinfektion forskelligt

- To disinfect: to destroy or irreversibly inactivate specified infectious fungi and bacteria, but not necessarily the spores, on hard surfaces.
- To sanitize: to reduce microorganisms of public health importance to levels considered safe, based on established parameters, without adversely affecting either the quality of the product or its safety.

- I industrien vil også skadevoldende ell. kvalitetsforringende mikroorganismer indgå

Desinfektionsmåder, eksempler

" Fysiske"

- Varme
- UV
- Bestråling
- Ethylenoxid
- Ozon
- Cold Plasma
- Ultralyd
- Filtrering (væsker)

"Kemiske"

Oxiderende, reactive

- Hypoklorit
- Pereddikesyrer (PAA)
- Brintoverilte
- Iodofor
- klordioxid

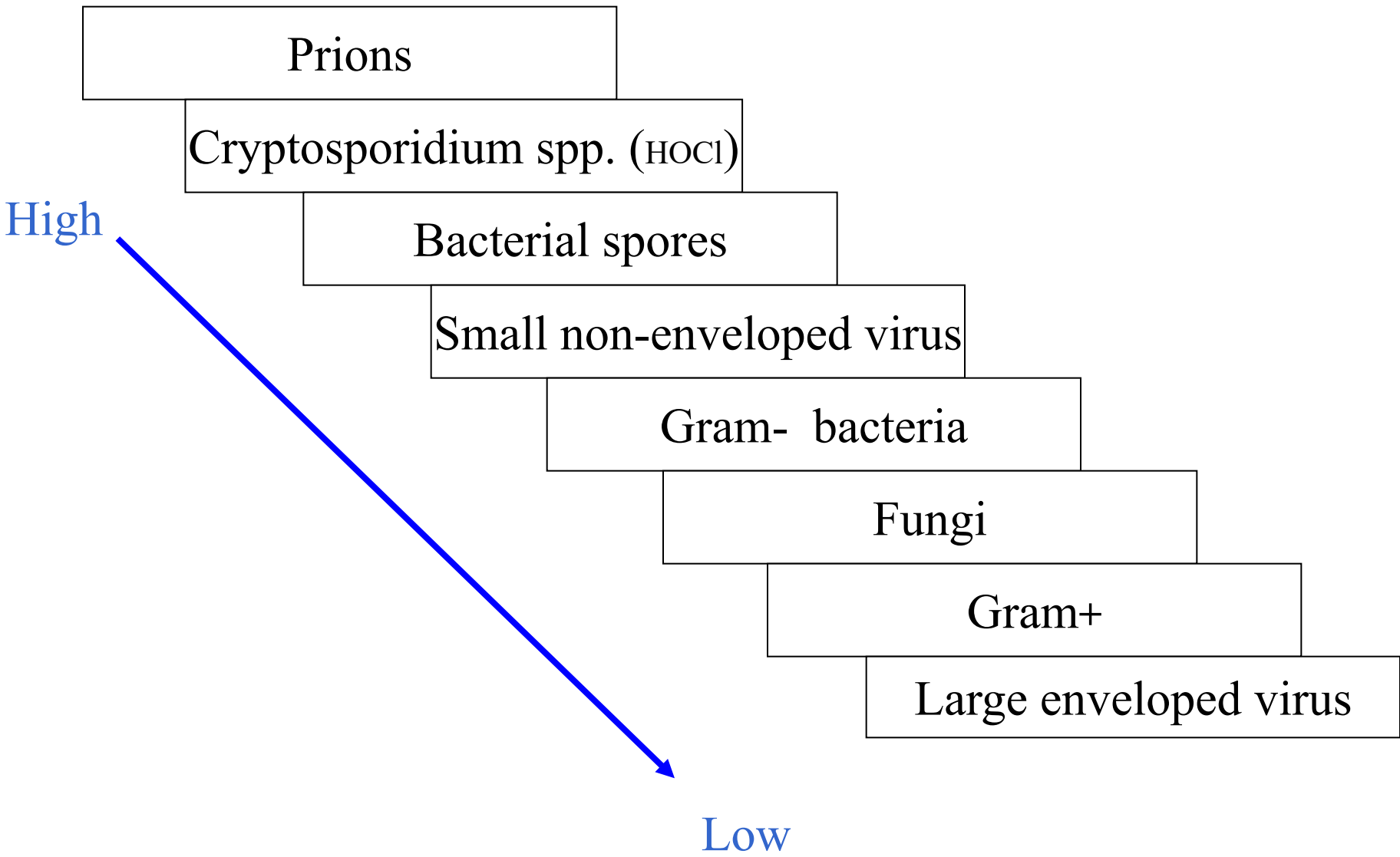
Stabile

- kvaternær ammonium (QAC)
- amfotære tensider
- Alkoholer
- Aldehyder

Hvilke kriterier vælger man efter?

- Effektivitet?
- Target organismer?
- Materialefølsomhed (corrosion mm)?
- Arbejdsmiljø?
- Toxiske rester i produkter (fødevarer, vand mm.)?
- Pris?
- Nemhed i brug?

General intrinsic biocide resistance



Heat

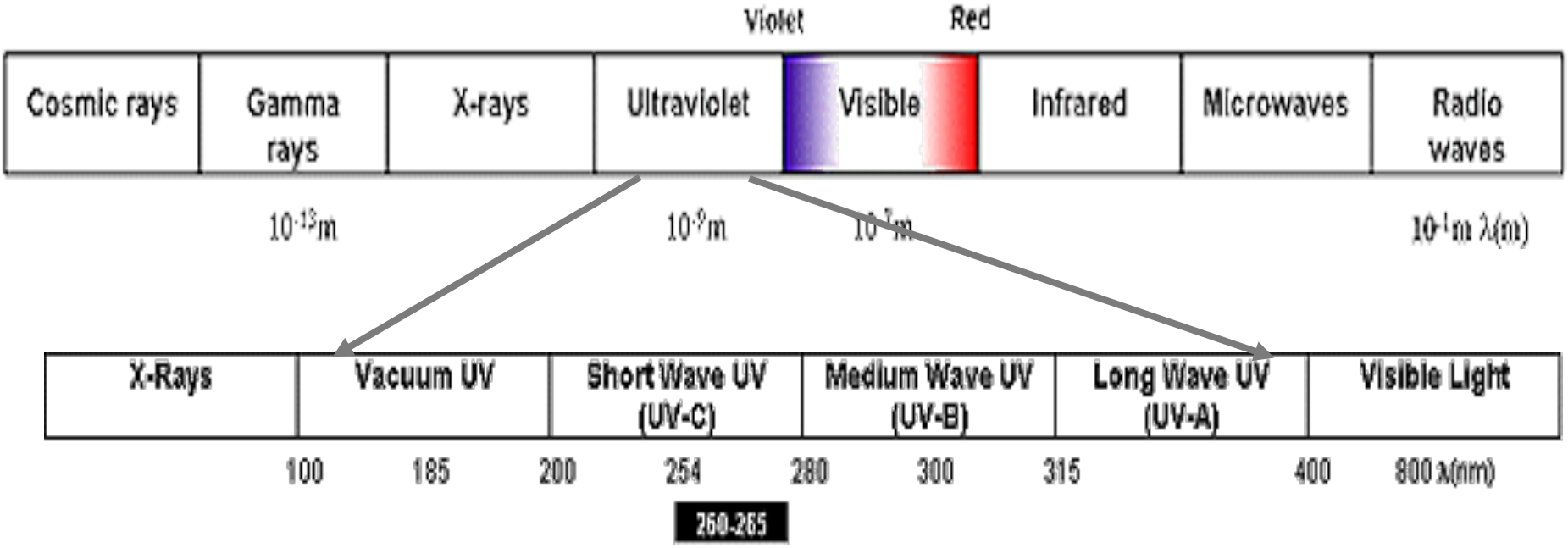
Incineration	>500^{0}C	vaporisation
autoclaving	121^{0}C, 15min	Sterilisation
Dry heat	160^{0}C, 2 hours	Sterilisation
Boiling	100^{0}C	Kills vegetative cells, many spores and toxins
Pasteurisation	65^{0}C, 30 min	**Reduces or inactivates most vegetative bacteria and yeasts but not spores**
Pasteurisation	72^{0}C, 15 sec	**do**

UV

- Used for water and surface disinfection
- Damages DNA
- User friendly, low cost
- Relatively short contact times
- Little surface penetration
- Photoactivation and ”dark repair” may occur
- Lamp fouling and shadow problems
- Will low pressure lamps (254nm) be replaced by LED lamps of different wavelengths?

UV er ikke ”bare” UV. Området er inddelt i 4 underområder: Vacuum UV, UV-C, UV-B og UV-A. Standard-enheden er nanometer (nm).

Electromagnetic Spectrum

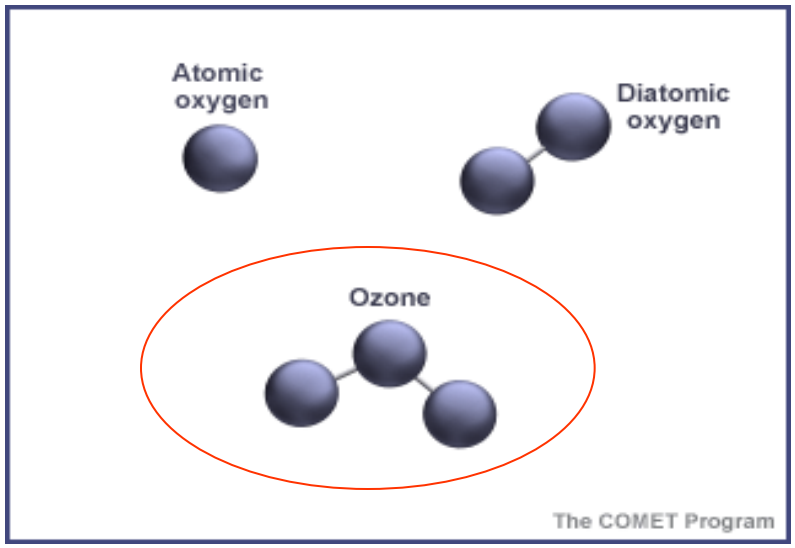


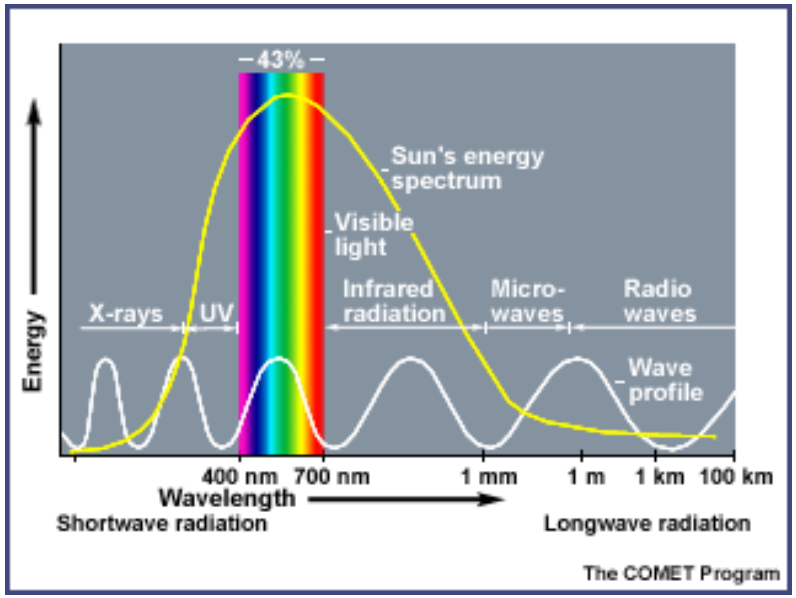
UV-B (280-315 nm) og UV-C (200-280 nm) er mest microbiocide

Idag: Lavtryks kviksølvlamper m. 254nm (primært dimer dannelse)

I fremtiden?: LED lamper m. 265nm (nucleotide abs. Max.) og 280nm (protein abs. Max)

Ozone





- Very strong oxidizing agent
- Effective with short contact time
- Unstable, generation on-site
- Mainly used for water
- Oxidizes cell wall and other components, radical formation during decomposition
- Highly corrosive
- Operator safety issues
- Absorbs UV-C and UV-B

kemiske desinfektionsmidler

adskiller sig fra antimikrobielle terapeutika bl.a. ved:

- Bredere og mere uspecifikt angrebsspektrum
- Anvendes i høje koncentrationer
- Hurtigtvirkende
- Anvendes i miljøet (f.eks. overflader på udstyr, rum, procesvand etc.)

Vigtige parametre for effektivitet

Rene overflader – langt bedre end overdosering

Kontaktsikkerhed

Temperatur

Koncentration

Kontakttid

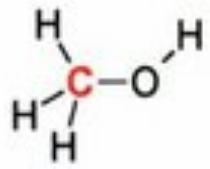
Type af mikroorganisme

Vandets sammensætning/kvalitet

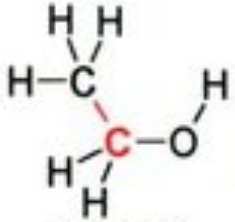
Biofilm

pH

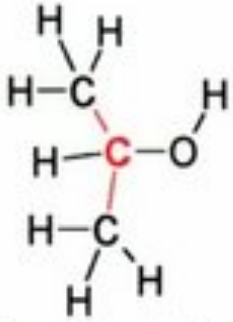
Alcohols



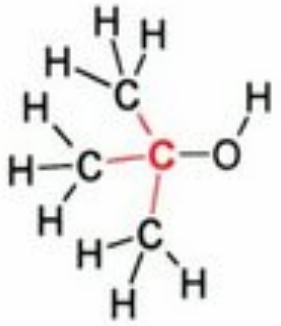
Methanol
also written
CH_3OH



Ethanol,
a 1° alcohol,
also written
CH_3CH_2OH



Isopropanol,
a 2° alcohol,
also written
$(CH_3)_2CHOH$



tert-Butanol
(2-Methyl-2-propanol),
a 3° alcohol, also
written $(CH_3)_3COH$

- Ethanol more efficient than isopropanol for virus
- Membrane disruption of cells (fluidity and leakiness)
- Flammable
- No specific resistance observed
- Little effect on spores, limited on some naked virus
- Conc. from 70-90% more efficient. For some viruses acid mix

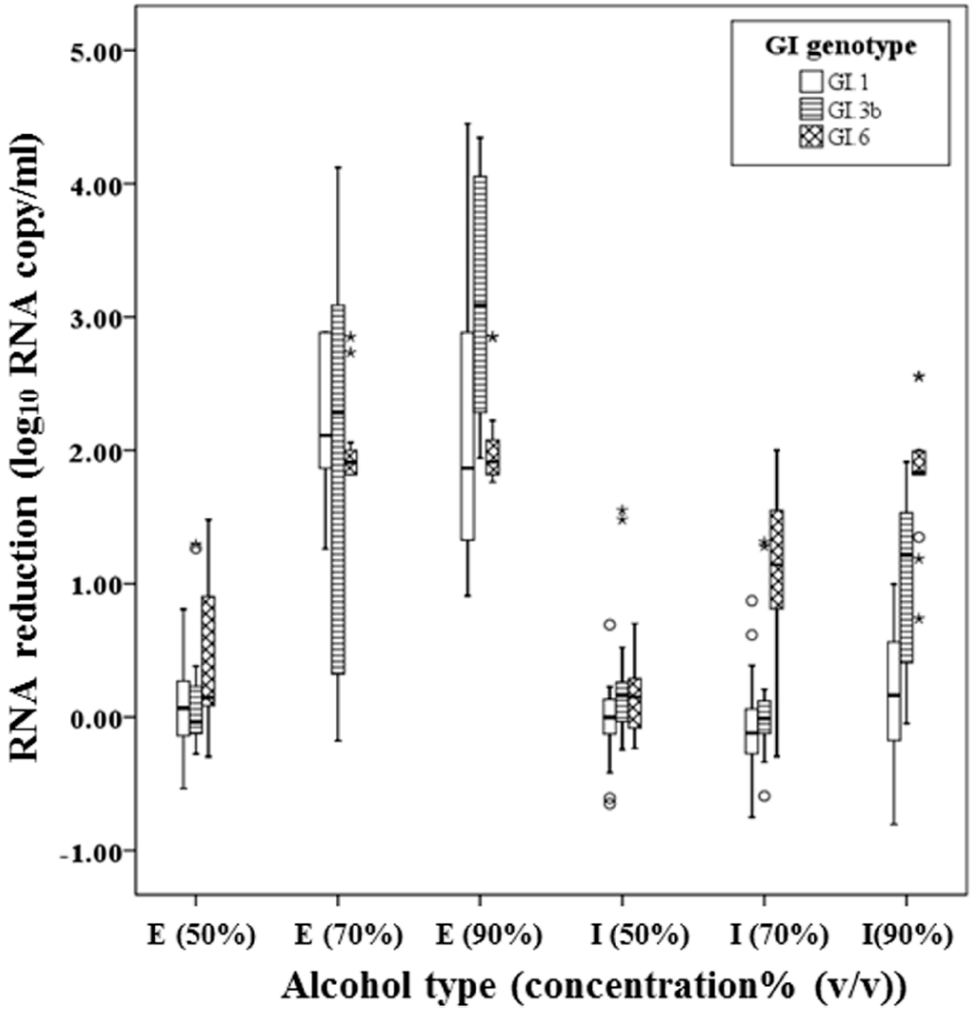


Fig 1. Virolysis patterns of GI.1, GI.3b and GI.6 norovirus strains after exposure to ethanol (E) and isopropyl alcohol (I) at 3 different concentrations (50%, 70%, and 90%). 3 GI.1, 4 GI.3b and 4 GI.4 strains with three replicate per each were consolidated by genotype and were expressed as a box plot. The upper, lower ends of the box and the horizontal line in the box indicate the first (Q1), third quantiles (Q3) and median value of all data, respectively. The lower and higher ends of whiskers indicate the minimum and maximum value of all data, respectively. Isolated data points are outliers.

doi:10.1371/journal.pone.0157787.g001

Reductions as a function of norovirus genotypes, alcohols and conc.

(Park et al. 2016)

1. Little effect of 50%
2. Ethanol far more efficient
3. Genotypic differences

Kampf (2018) describes reports that find 1) good reduction at <70% when alcohol is mixed with specific acids and 2) that the common surrogate MNV is more sensitive than human norovirus

Klor

Produkt og overflade	Mikroorganisme	pH	Indvirkningstid Ved 20 -25 °C	Koncentration (ppm)
Natriumhypochlorit Rene overflader	De fleste bakterier[2,6,12,15]	7-9	≤ 10 min	100*
	Virus[6]	8-9	≤ 10 min	200*
	Mykobakterier[9]	8-9	20 min	1000
	Norovirus[10] "Roskildesyge"	8-9	10 min 1-3 min	500 1000
	Clostridium difficile sporer[3,6]	5-6 7-9**	10 min	1000 HOCl 5000
	Candida species[16]	?	30 sek	500
Dichlorisocyanurat Urene overflader	MSSA[13] MRSA	5-6	1 min 2½ min	1000 500

Hypochlorit i vandig opløsning:

- HOCl + OH- OCl- + H+ + OH
- Ved pH =5 er al OCl- omdannet til det aktive HOCl
- Ved pH > 10 er alt omdannet til det næsten inaktive OCl-
- Ved pH < 3 omdannes HOCl til giftigt frit klor og vand

Reaktivt klor

Ofte brugt i andre lande i vandbehandling og til dekontaminering af fødevarer

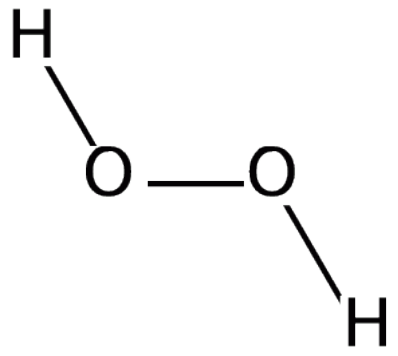
Hyppigt anvendt til desinfektion af udstyr

Effekt pH afhængig (pH 5-7), inaktiveres af organisk materiale

Lugter (og danner kraftige lugte som trichloroanisoler med skimmel, dvs. uønsket i vinkældre)

Reagerer med (I rækkefølge)

- 1. svovlholdige aminosyrer som cystein og methionin
- 2. med aminer
- 3 Lipider og nukleotider



Hydrogen peroxide

- Radical formation
- Unstable
- No specific resistance observed
- High catalase activity may be induced
- Differences observed between liquid and gaseous form (gas being more potent)

Peroxyacetic acid

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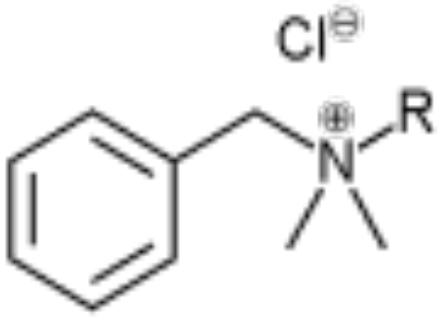
- Metal ion sensitivity
- Corrosive to soft metals
- Odour of concentrate
- Varied activity against fungi

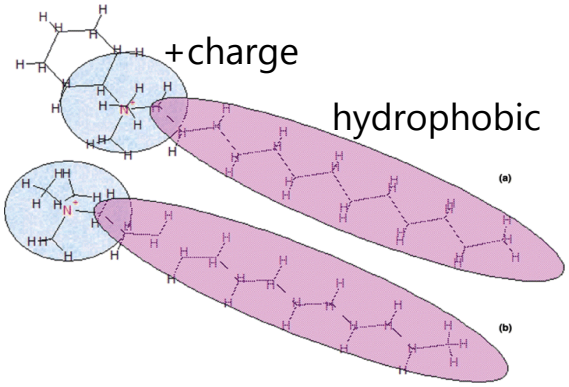
\+

- Low foam
- Broad temperature range of activity
- Combine disinfection and acid rinse
- No residue
- Generally non – corrosive to stainless steel and aluminium.
- Relative tolerance to organic soil
- Phosphate free
- Environmentally responsible
- Broad spectrum of bactericidal activity
- Active over broad pH range up to pH 7.5

Quarternary Ammonium Compounds (QAC)

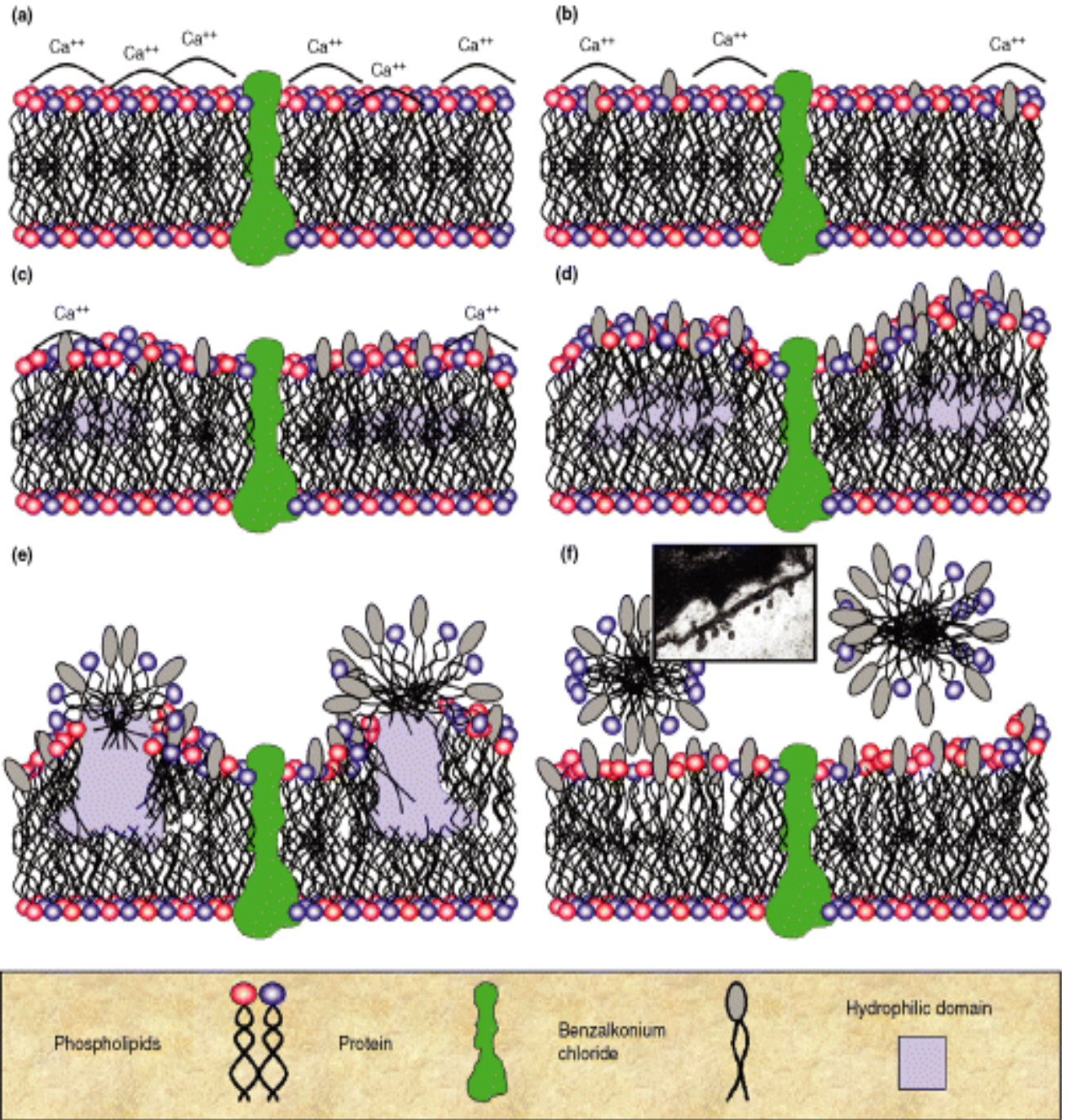


$R = C_8H_{17} - C_{18}H_{37}$



Benzalkonium Chloride, Cetrimide

- Hydrophobic/hydrophilic
- Surface cleaning and disinfection (equipment, floors etc.)
- Surfactants, interferes with the cell membrane
- Many variants and formulations developed
- Affected by soiling, soaps and hard water
- Increased tolerance observed in grampos. and gramneg.
- Resistance due to different types of efflux pumps



QAC

adsorption of the quaternary headgroup to acidic phospholipids in the membrane

decreased fluidity of the bilayers and the creation of hydrophilic voids in the membrane

solubilization of phospholipids and proteins into QAC/phospholipid micelles

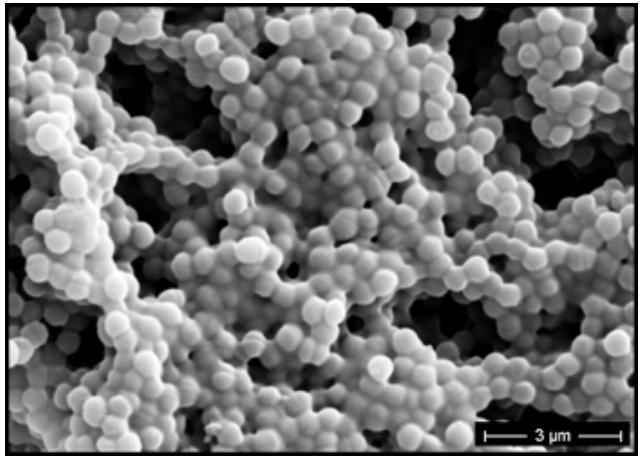
Why do we see reduced inactivation/increased tolerance to biocides in the processing industry?

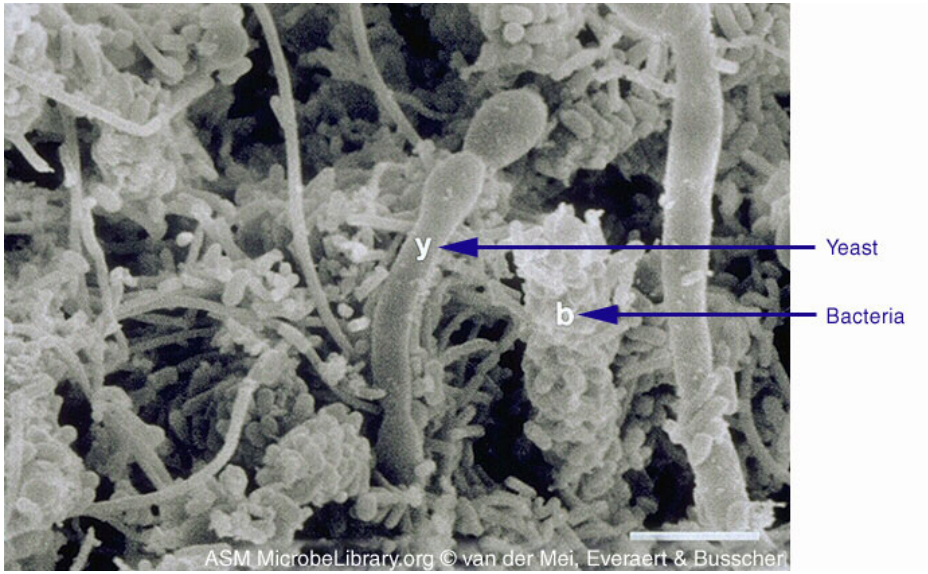
Physiological changes

Matrix related

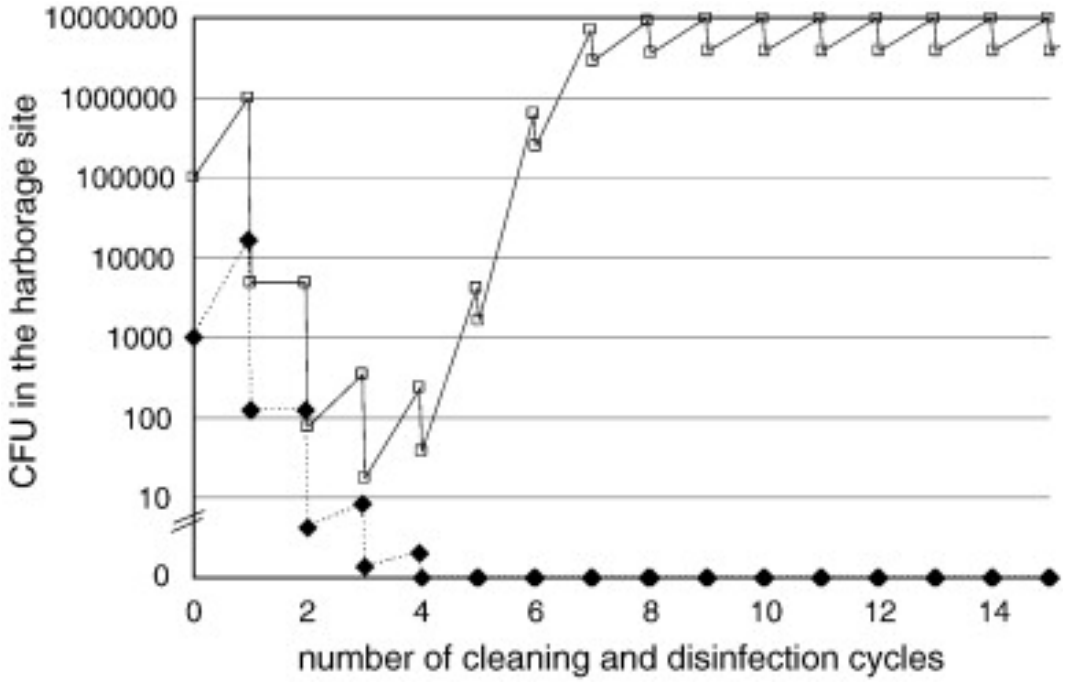
Genetic changes

Matrix: Microorganisms in aggregates/on surfaces embedded in a matrix =biofilm





Biofilm



Clean

Evolution model of the viable cell density of a bacterial strain in a harborage site contaminated once and submitted to cleaning and disinfection at regular time intervals.

Brigitte Carpentier, Olivier Cerf

International Journal of Food Microbiology, Volume 145, Issue 1, 2011, 1–8

http://dx.doi.org/10.1016/j.ijfoodmicro.2011.01.005

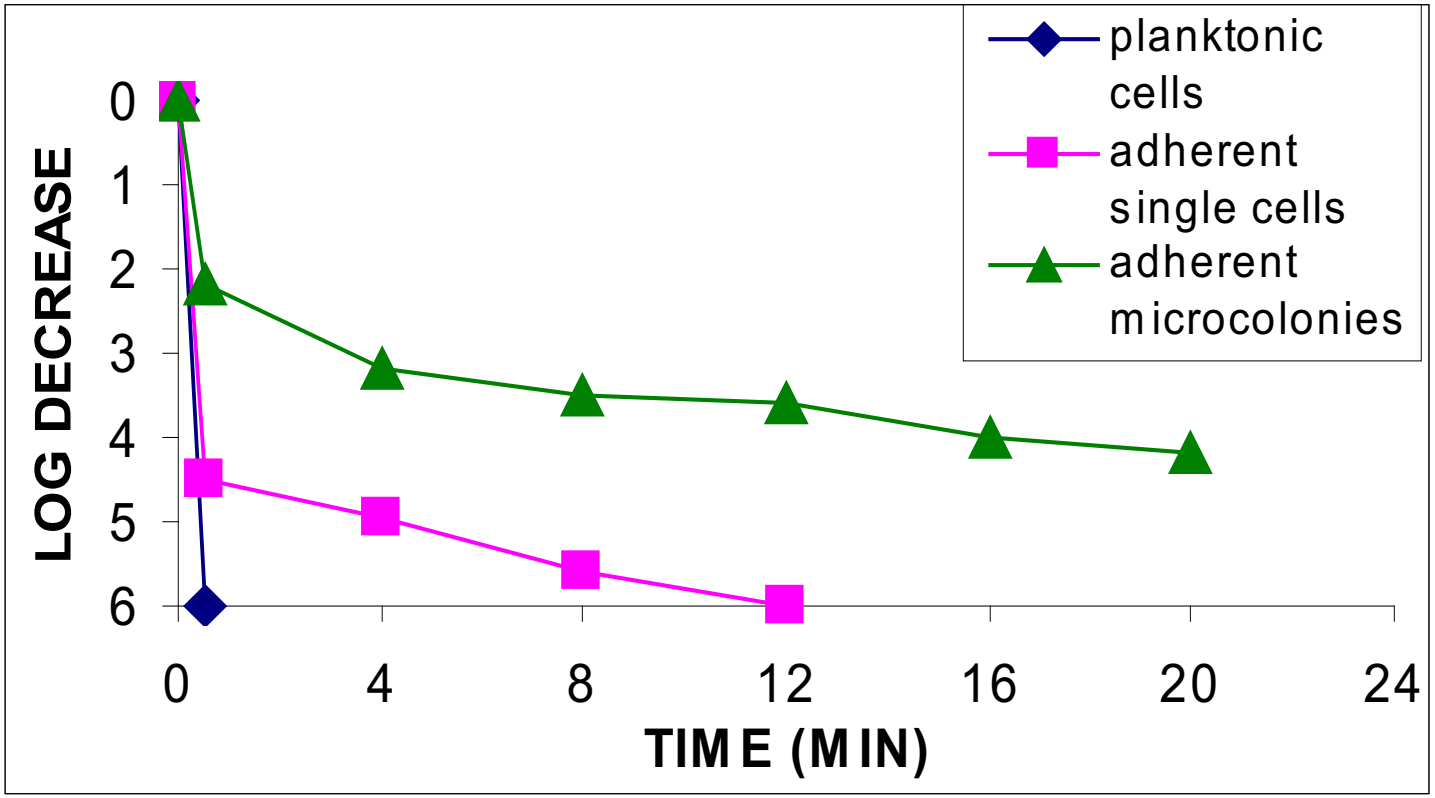
Early realization that biofilm decreases suceptibility

Ratio MBC biofilm / MBC planktonic cells for *Pseudomonas aeruginosa* (from Ntsama *et al.*, 1995)

Quaternary ammonium compounds		Amphoteric	Oxydants		Phenolics	
Cetrimide	Benzalkonium chloride	Tegol®	Peracetic acid	Sodium Hypochlorite	Phenol	O-Cresol
> 400	100	25	4	5	1	4

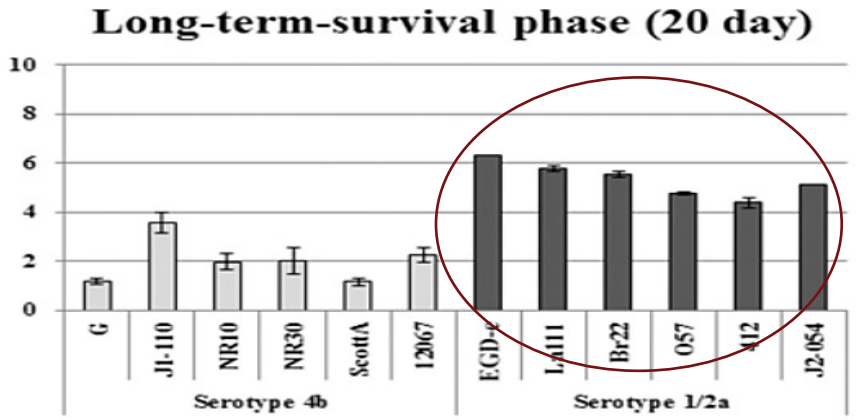
MBC : minimal bactericidal concentration is the concentration necessary to obtain 5 decimal reductions within 5 minutes at 20°C

Role of surface attachment for treatment of ***Listeria monocytogenes*** by 800 mg/l benzalkonium chloride (Frank et Koffi, 1990)

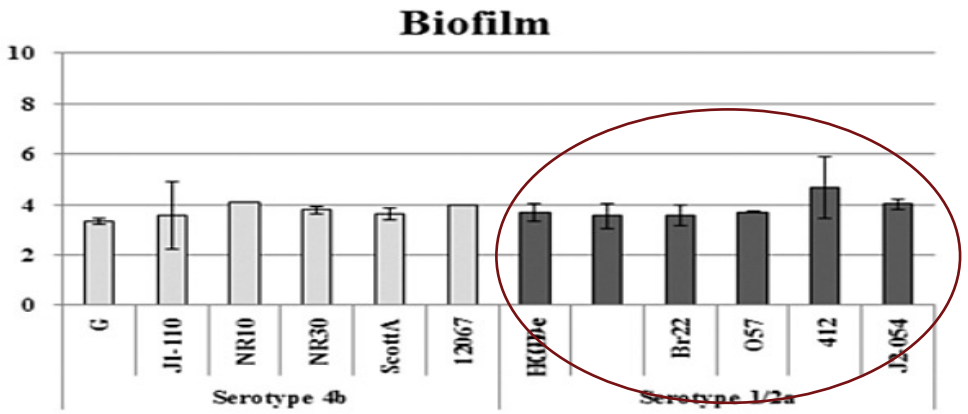


Listeria monocytogenes susceptibility to H_2O_2: effect of biofilm & serotype (1/2a and 4b)

0.6%, 10min



5%, 10min



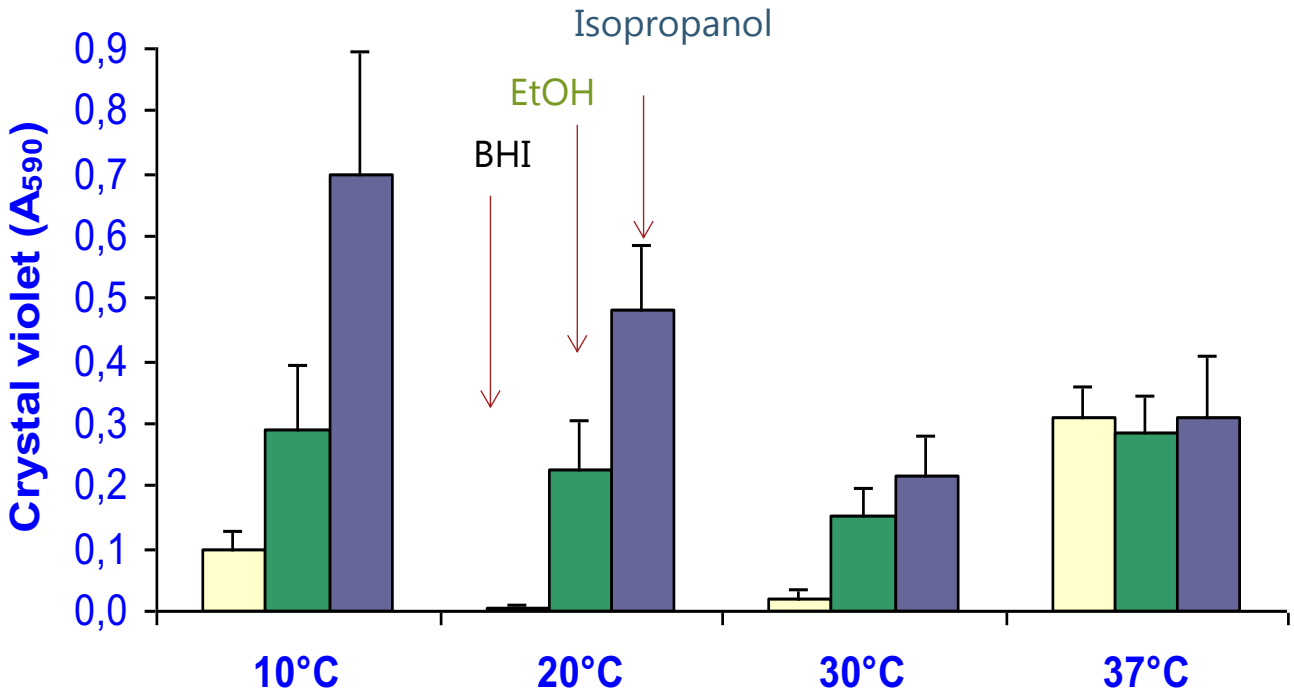
Reduction of *L. monocytogenes* serotype 4b strains (light gray bars) and serotype 1/2a strains (dark gray bars) after treatment with H_2O_2.

Bacteria were grown in BHI broth at 37 °. Cells of long-term-survival phase (20 days) were treated with 0.6% H_2O_2 for 10 min). Biofilm cells were treated with 5% H_2O_2 for 10min. Plate counts were carried out in triplicate on at least two biological replicates

Huang et al. Food Control, 85(2018)

Subinhibitory concentrations of biocide may stimulate biofilm formation

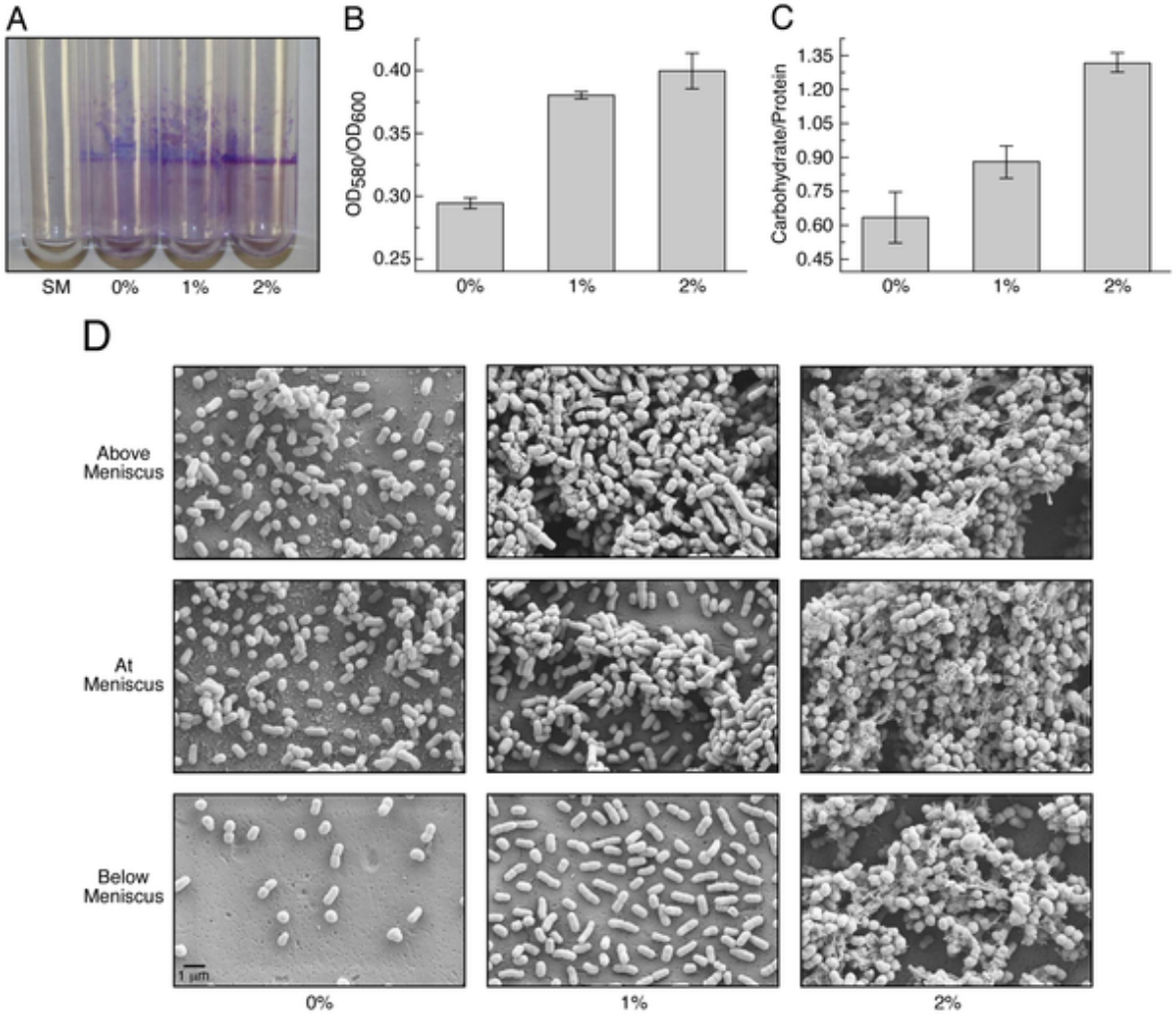
Example: Low alcohol induces surface attachment/biofilm in *Listeria*



Surface attachment of *L. monocytogenes,* EGD, grown in BHI (White), BHI with 2,5% ethanol (Green) and BHI with 2,5% isopropanol (Blue) at different temperatures

Gravesen, Lekkas, and Knøchel (2005), *Appl. Environ. Microbiol.*, 71, 5601-5603

Effects of low ethanol conc. on biofilm formation and carbohydrate production of *Acinetobacter baumannii*.



Nwugo CC, Arivett BA, Zimbler DL, Gaddy JA, Richards AM, et al. (2012) Effect of Ethanol on Differential Protein Production and Expression of Potential Virulence Functions in the Opportunistic Pathogen Acinetobacter baumannii. PLOS ONE 7(12): e51936. https://doi.org/10.1371/journal.pone.0051936
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0051936

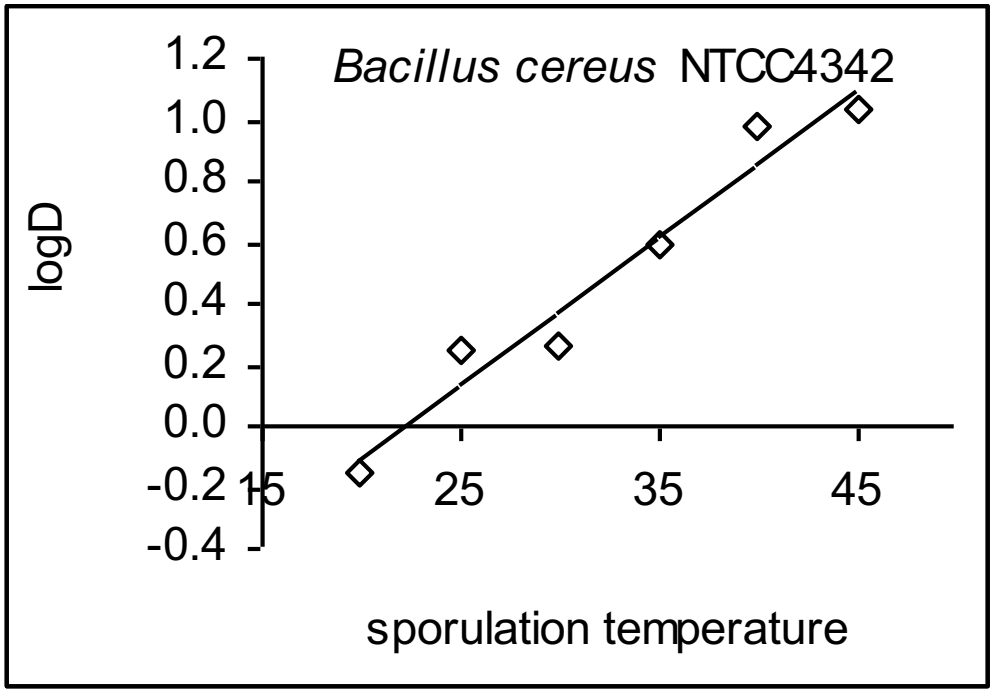
PLOS ONE

Adhering spores of *Bacillus cereus* more heat resistant

Decimal reduction time	D_{90} (min)		D_{110} (min)	
	Planktonic	Attached	Planktonic	Attached
B. cereus A	1.59	3.87		
B. cereus B	2.13	5.82		
B. stearotherm. A			1.95	5.12
B. stearotherm. B			1.15	2.79

Simmonds et al. 2003

Spores of *B. cereus* formed at high temperatures are more heat resistant



B cereus 4342, Heating temperature 98°C
(Gonzales et al. 1999)

Antimicrobial susceptibility and biofilm interactions

Susceptibility changes:

- Planktonic cells << adhered cells << mature biofilm
- Multispecies more robust than monoculture
- Biofouling may further decrease susceptibility
- Spores more robust when adhered
- Spores may be more heat resistant when formed in warm biofilms

Genetic transfer increases in frequency and host range in biofilm

Subinhibitory biocide stress may increase biofilm formation

- Rengøring er stadig det vigtigste – og en forudsætning for effektiv desinfektion
- Desinfektion nødvendig, hvor overførsel af patogener eller andre skadevoldere kan være problematisk
- Stor variation i tolerance ikke bare mellem forskellige typer organismer, men også mellem individuelle genotyper og graden af adaptation
- Variationer i desinfektionen (formulering, tilstandsform, bølgelængder mm.) kan ændre effektivitet, så gamle "tommelfingerregler" skal løbende revideres
- Mange parametre influerer effektiviteten
- Hvordan finder vi balancen mellem den nødvendige desinfektion og ønsket om at reducere sygdom, fordærv og teknisk, mikrobielle problemer samt energi-, tids- og kemikalieforbrug og økonomiske, miljømæssige og arbejdsmiljømæssige hensyn?