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A Concept Of A Probe For Particle Analysis And Life Detection In Icy Environments

Amateur´s Opinion

Points of Interest:

- Ice Penetration
- In-situ Decontamination
- In-situ Detection of Bacteria

Ice Penetration

- Conventional Drilling
- Autonomous Drilling
- A Melting Probe
- A Melting/Drillig Probe

In-situ Decontamination:

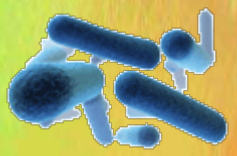
- Guideline: Planetary Protection
- Criteria
- Consequent Selection
- Results

Our criteria for a proper in-situ decontamination method:

- High killing rate and activity against broad range of microorganisms, spores, fungi, viruses, small insects and worms (sic!)
- Fast decomposing and environmental „friendly“ end products
- Good solubility in water
- Good penetration ability
- Activity in relatively cold environments
- Easy storage, handling and application
- Cost
- Better: a subsequent application of 2-3 compounds (!)

Antiseptics and disinfectants

- **Acridine derivatives** Ethacridine lactate · Aminoacridine · Euflavine
- **Biguanides and amidines** Dibrompropamidine · Chlorhexidine · Propamidine · Hexamidine · Polihexanide
- **Phenol and derivatives** Hexachlorophene · Policresulen · Phenol · Triclosan · Chloroxylenol · Biphenylol
- **Nitrofuran derivatives** Nitrofurazone
- **Iodine products** Iodine/octylphenoxypolyglycolether · Povidone-iodine · Diiodohydroxypropane
- **Quinoline derivatives** Dequalinium · Chlorquinaldol · Oxyquinoline · Clioquinol
- **Quaternary ammonium compounds** Benzalkonium · Cetrimonium · Cetylpyridinium · Cetrimide · Benzoxonium chloride · Didecyldimethylammonium chloride
- **Mercurial products** Mercuric amidochloride · Phenylmercuric borate · Mercuric chloride · Mercurochrome · Thiomersal · Mercuric iodide
- **Silver compounds** Silver nitrate
- **Alcohols** Propanol (Propyl Alcohol · Isopropanol (Isopropyl Alcohol) · Ethanol (Ethyl Alcohol) ·
- **Other** Potassium permanganate · Sodium hypochlorite · Hydrogen peroxide · Eosin · Tosylchloramide sodium **.....**



Common pesticides with established efficacy (EPA standards)

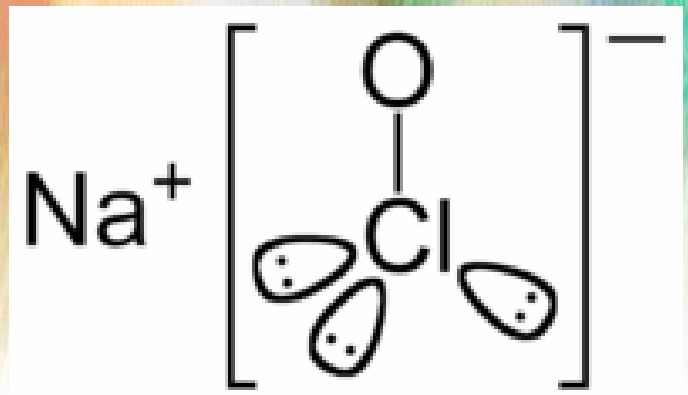
- Bleach (sodium hypochlorite)/chlorine dioxide
- Ethylene oxide,
- Hydrogen peroxide
- Peroxyacetic acid,
- Methyl bromide,
- Formaldehyde/paraformaldehyde
- Perborate (“persil“)
- Percarbonate/pseudopercarbonate

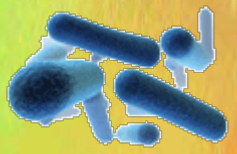
Boron contamination

Bleach (sodium hypochlorite)

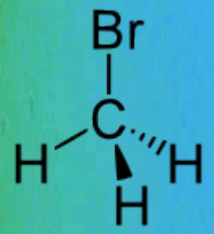
- Chlorination of water (swimming pool)
- + Is allowed for food processing equipment: A weak solution of 1% household bleach in warm water is used to sanitize smooth surfaces prior to brewing of beer or wine

- - corrosive





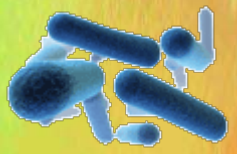
Methyl bromide



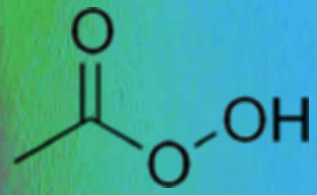
- + Safe and efficient soil sterilant
- + occurs naturally in the ocean, where it is found in some species of algae

- - its use is curtailed by the Montreal Protocol

(the list of banned ozone-depleting substances)



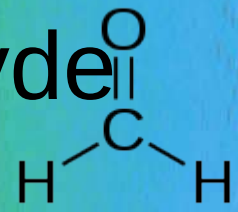
Peroxyacetic acid



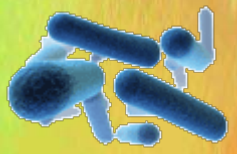
- + very efficient and fast antimicrobial agent ($OH^{\cdot}$)
- + no resistant microorganisms exist
- + can damage virtually all types of macromolecules associated with a microorganism: carbohydrates, nucleic acids, lipids and amino acids = no organic contamination
- + end products: acetic acid (!) and hydrogen peroxide (!)
- very effective at low temperatures

- - highly corrosive
- - being pure can explode at elevated temperatures

Formaldehyde/paraformaldehyde



- Saturated water soution = formaline $H_2C(OH)_2$
- +/- Environmental concerns
- - Toxic, allergic, carcinogenic
- - Variety of chemical reactions
- Kills most bacteria, fungi (including their spores), and parasites. Also used in vaccines
- +/- Preserves/inactivates organic molecules by cross-linking

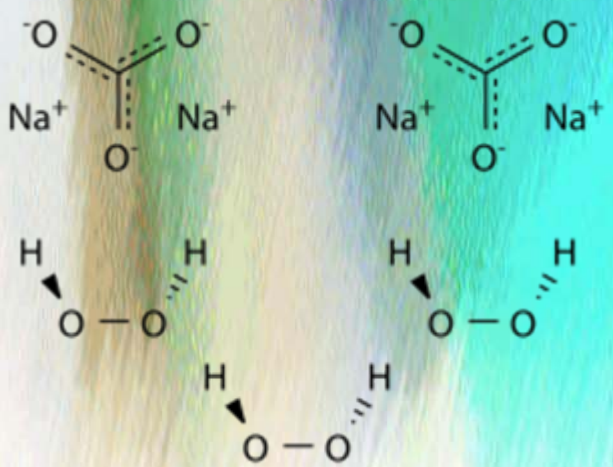


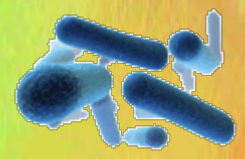
Ethylene oxide

- + well-studied bactericidal effects, „standard“
- - end product in reaction with water is ethyleneglycol and ist derivatives
- - gaseous
- - potentially carcinogenic

Percarbonate/pseudopercarbonate

- True percarbonates MeO-CO-O-OH are highly unstable
- “Sodium percarbonate” is in reality a mixture 2 $Na_2CO_3 \cdot 3\ H_2O_2$
- +/- solid
- - unstable at 60°C
- - creates very alkaline pH

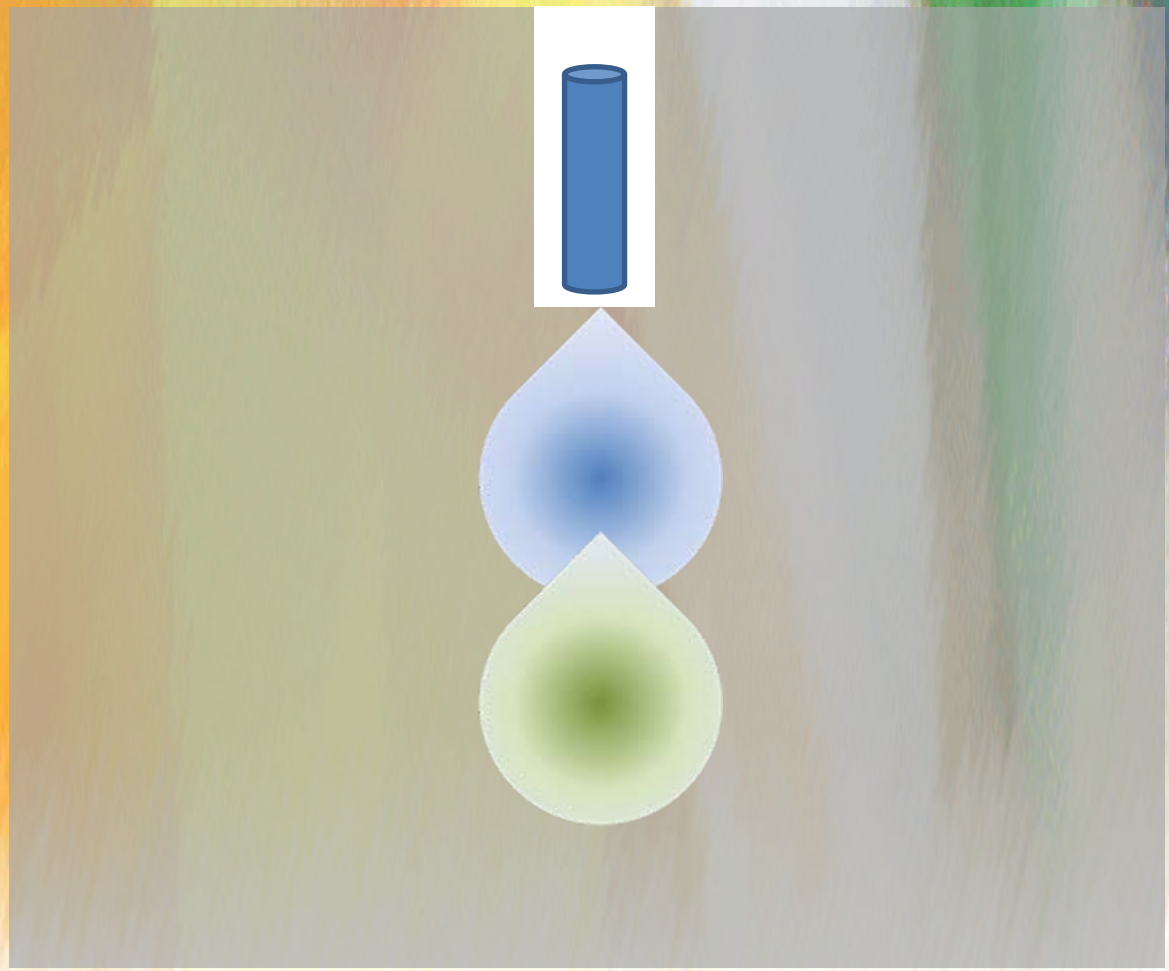


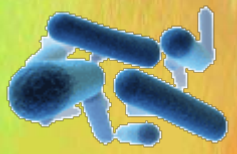


Hydrogen Peroxide H_2O_2

- + Can operate at low temperatures
- + Humidity level is not as critical as with formaldehyde
- + Is residue free
- + Has fast “kill” times
- + Is a fully validated technology
- - microorganisms having catalase or peroxidase have a chance to survive
- - ?

In situ Decontamination: a Car Wash Approach





Suggestion:

Two-step in-situ decontamination process is suggested, consisting of a consequent application of two of the following compounds:

1. Sodium hypochlorite
2. Peroxyacetic acid/Hydrogen peroxide
3. Methyl bormide
4. Sodium percarbonate

Life Detection:

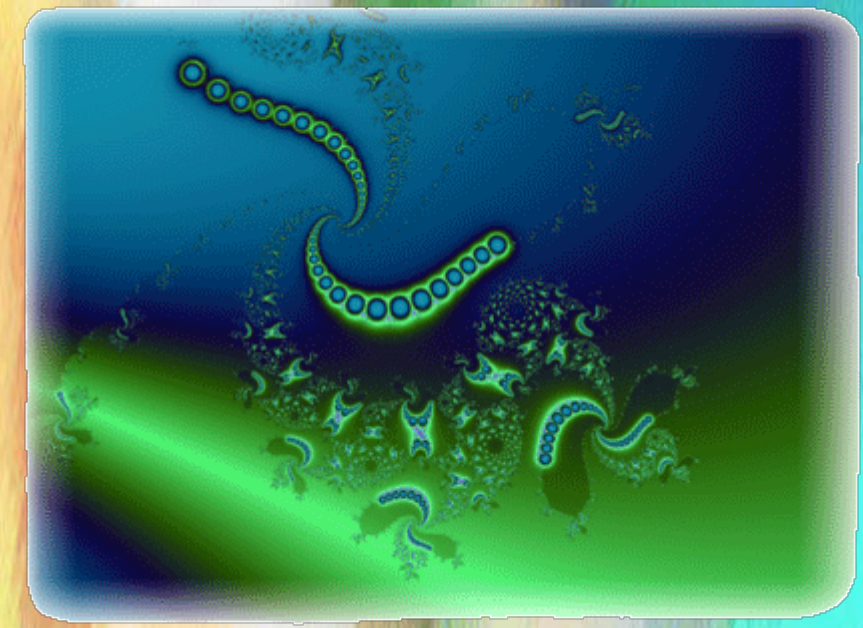
Challenge:

- rapid
- in-situ
- non-invasive

method for detection, quantification and identification of microscopic organisms in water and glacial environments

Approaches Evaluated:

- Autofluorescence
- Light Scattering
- Flow Cytometry



Autofluorescence:

- +

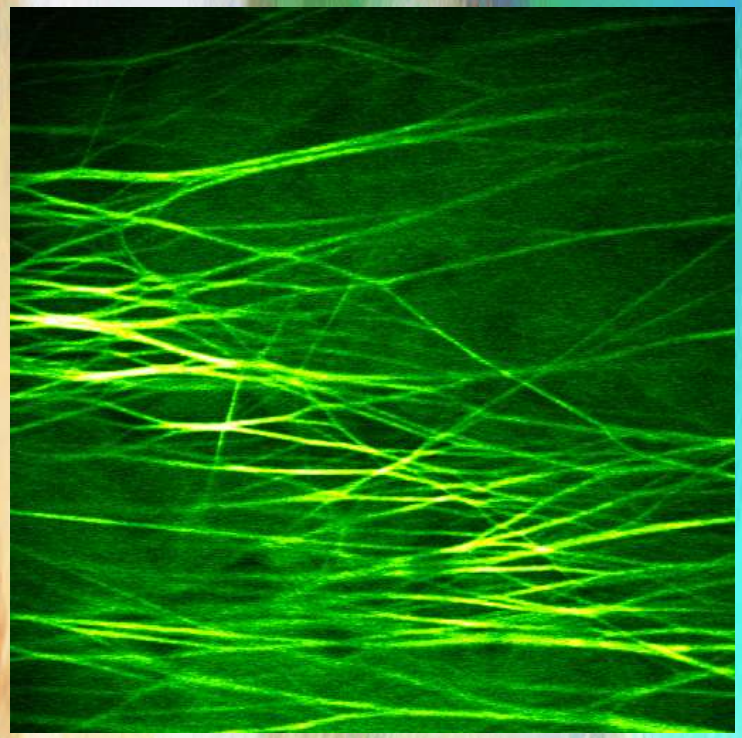
  Non-invasive

  Easy to apply

- -

  Low specificity

  Weak signal



Autofluorescence may carry useful biological information as well:

- NADH can be used to monitor metabolic state of living tissues and for redox fluorometry, redox NAD(P)H imaging
- flavoprotein may be used for imaging and redox state detection of intracellular flavins using flow cytometry or fluorescence microscopy
- FAD fluorescence has been used for identification and cell sorting of eosinophils and neutrophils.

Fluorescent molecules naturally present in animal tissues

Fluorescent compound	Localization	Excitation maxima [nm]	Emission maxima [nm]
Aromatic amino acid residues:	cofactors in metabolism, concentrated to mitochondria, also present in cytoplasm		
Tryptophan		280	348
Tyrosine		274	303
Phenylalanine		257	282
Pyridine nucleotides (reduced)	cofactors in metabolism, concentrated to mitochondria, also present in cytoplasm		
NADH		290 351	440 460
NADPH		336	464
Flavins and flavin nucleotides (oxidized)	riboflavin, FMN and FAD, mostly bound to enzymes as coenzymes of flavoproteins, concentrated to mitochondria, also present in cytoplasm and outer membrane	≈223 ≈268 ≈374 ≈449	broad maximum around ≈530
Collagen	connective tissue	280 265 330 450	310 385 390 530
Elastin	connective tissue	290 320 350 410 450	340 405 420 500 520
Endogenous porphyrins	In erythroid cells	400-500	630 690
Lipofuscin	Pigment granules accumulated with age in various cells	≈360	≈450

Light Scattering:

- +

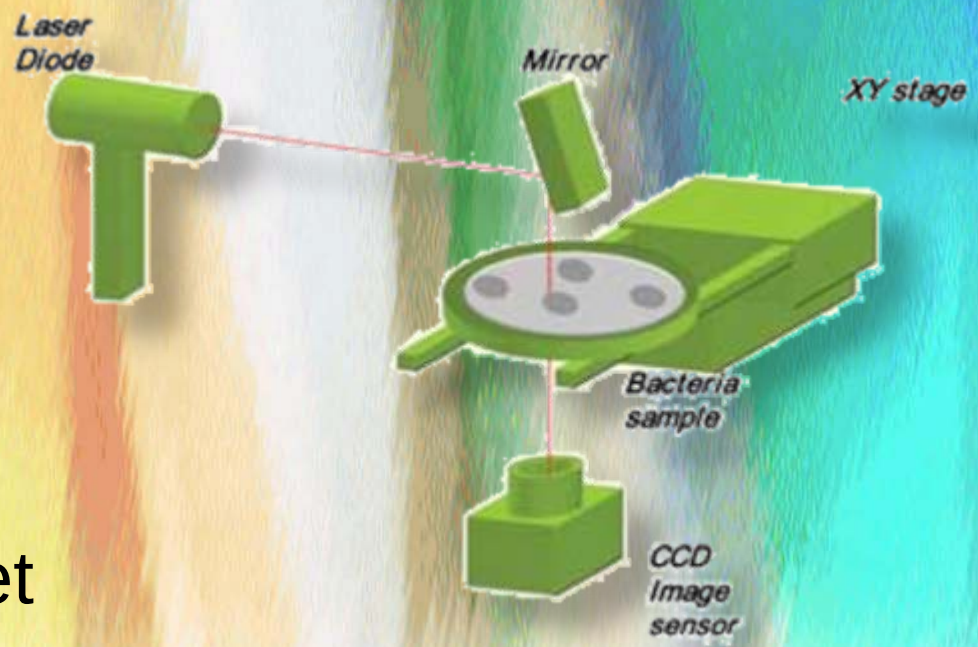
  Non-invasive

  Very sensitive

- -

  Noise

  Difficult to interpret



Flow Cytometry

In 1947 Gucker and colleagues: “The principle (of flow cytometry) should have wide application in (. . .) bacteriology.”

- +

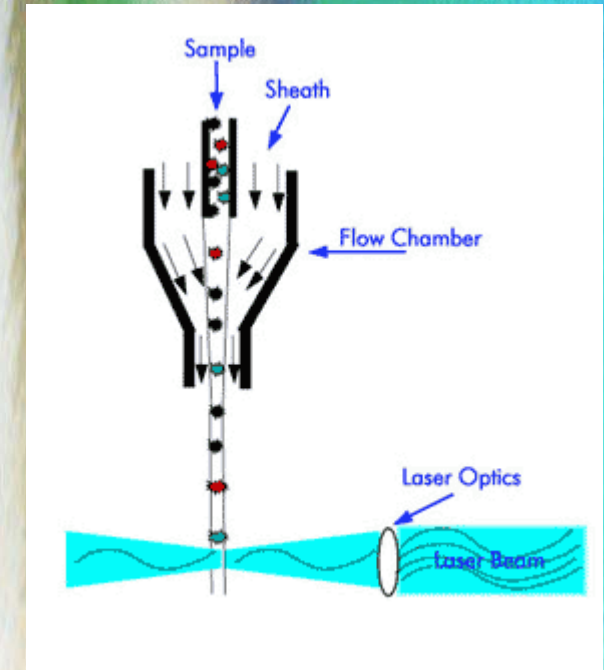
  Non-invasive

  Very sensitive (single cell counting)

- -

  Noise

  Difficult to interpret




Thank you