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Fraunhofer Institute for Organic Electronics, Electron Beam and Plasma Technology FEP

Hygienization with UV radiation

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Agenda

1. **Current relevance**
2. **Definitions**
3. **Germicidal efficacy**
   - Sensitivities and wavelength dependence
   - Damage to DNA/RNA (direct)
   - Damage via reactive oxygen species (indirect)
   - Effectiveness comparison of the wavelength ranges - an example
   - Combined application with photocatalysts
4. **Repair of (photo-induced) DNA damage**
   - Photoreactivation
   - Nucleotide/base excision repair (NER, BER)
5. **Comparison with other disinfection and sterilization methods**
6. **Generation of artificial UV radiation - advantages and disadvantages of LEDs**
7. **Current products, projects and research**
8. **Summary**

CURRENT RELEVANCE

- established technology for years in water and air disinfection, food sterilization, surface decontamination and disinfection of medical equipment
- in times of Corona the use has increased

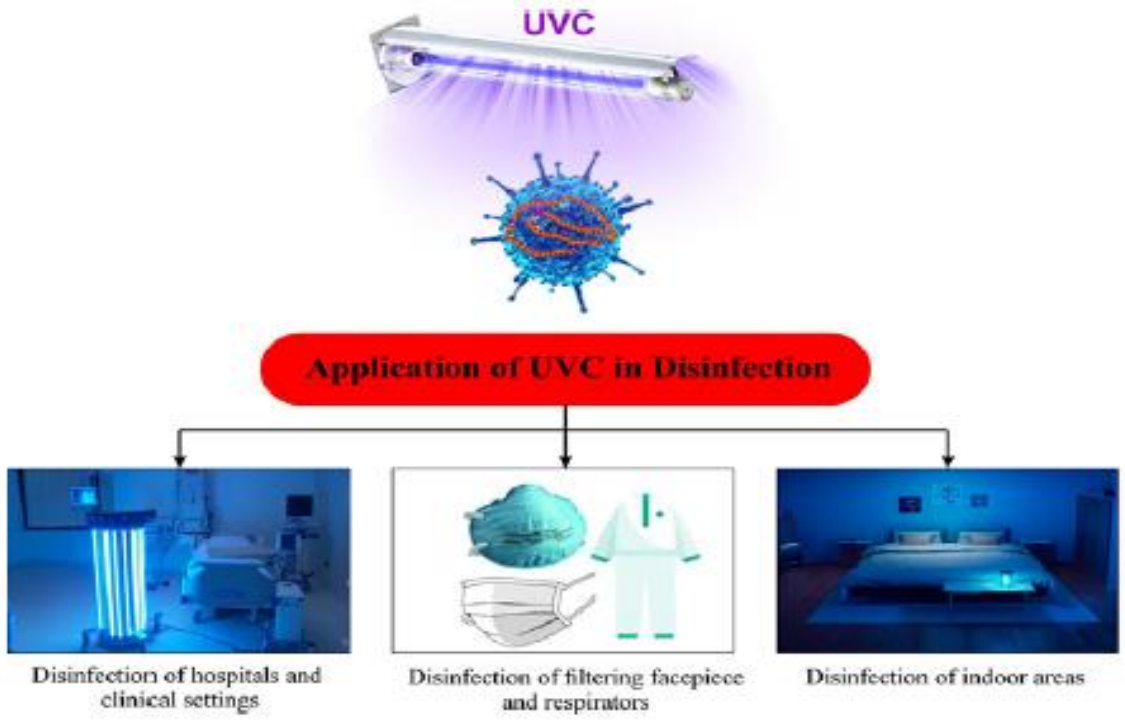

Fig. 1 & 2: Areas of application of UV disinfection [1]

[1] Bhardwaj et al., Science of the Total Enviroment, Volume 792, 148548 (2021)

DEFINITIONS

DEFINITIONS

LOG REDUCTION, DISINFECTION, STERILIZATION, INACTIVATION

log reduction: $log_{10}(\frac{initial\ germ\ concentration}{germ\ concentration\ after\ treatment}) = log_{10}(\frac{N_0}{N_t})$

log	reduction by … germs	in %	
1	10^1	90	
2	10^2	99	
3	10^3	99,9	
4	10^4	99,99	→ **disinfection**
5	10^5	99,999	
6	10^6	99,9999	→ **sterilization**
7	10^7	99,99999	
.	.	.	
.	.	.	
.	.	.	

inactivation: complete destruction of the biological activity of microorganisms and biological agents

DEFINITIONS
UV RADIATION

- most energetic part of optical radiation
- not visible to humans

- sources
  - natural: sun
  - artificial : LEDs, gas discharge lamps, lasers, etc.

- effects of UV radiation
  - acute: sunburn, conjunctivitis of the eye
  - chronic: aging of the skin, skin cancer, cataract, DNA damage

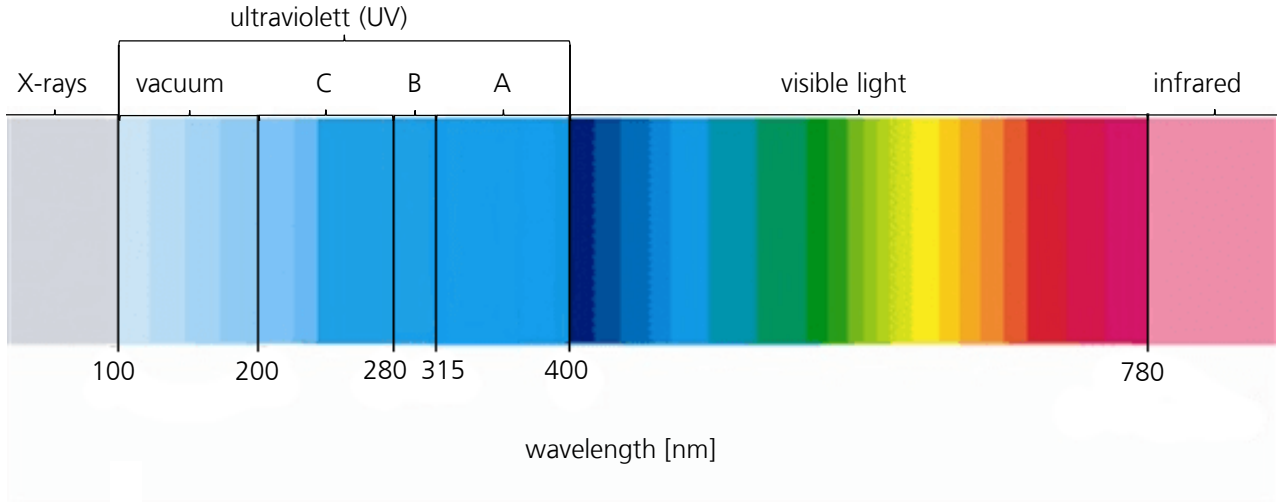

Fig. 3: Section from the electromagnetic spectrum

DEFINITIONS
UV RADIATION

classification according to wavelengths:

- UVA (near UV): 315 – 400 nm
- UVB (medium UV): 280 – 315 nm
- UVC (far UV) : 200 – 280 nm
- VUV (vakuum UV): 10 – 200 nm

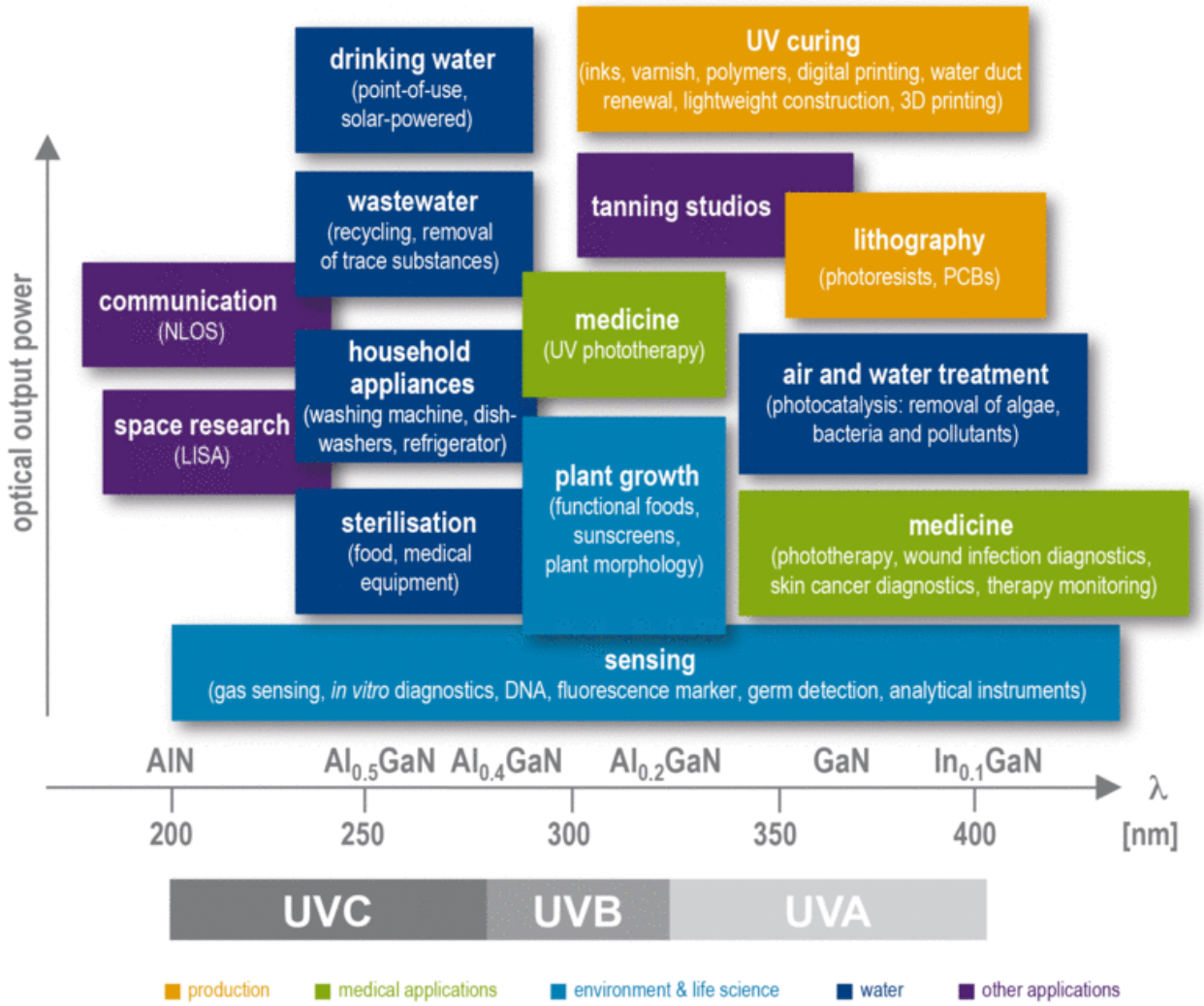

Fig. 4: Applications of UV radiation [URL-1]

[URL-1] Advanced UV for Life, 2022, URL: https://www.advanced-uv.de/en/uv-basics/uv-radiation (last access 01.03.2022)

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DEFINITIONS
UV RADIATION

$$applied\ dose[\frac{mJ}{cm^2}] = irradiance\ [\frac{mW}{cm^2}] \times irradiation\ time\ [s]$$

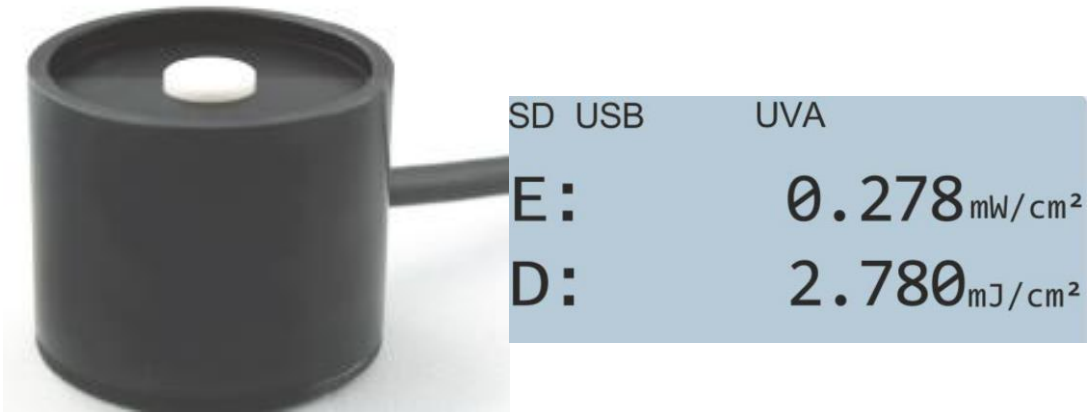

Fig. 5 & 6: UV radiometer (top) and UV-sensitive film dosimeter (bottom)

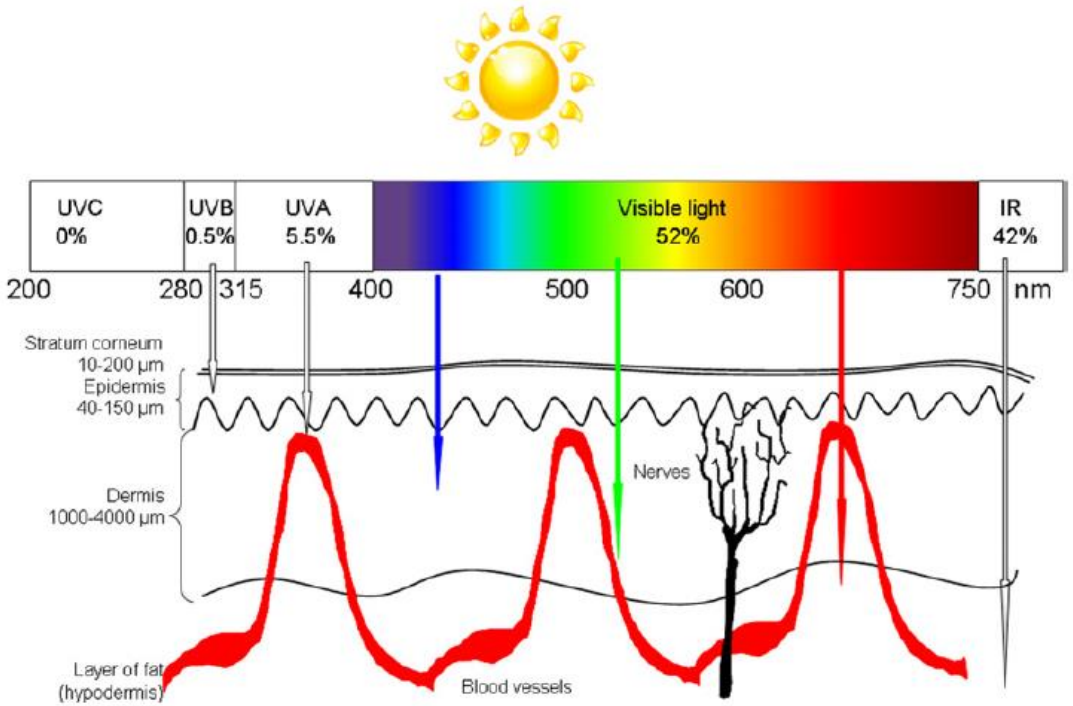

Fig. 7: Approximate depth for penetration of optical radiation in fair Caucasian skin [2]

- low penetration depth of a few mm to cm
- penetration depth increases with the wavelength

[2] Juzeniene et al., Reports on Progress in Physics, Volume 74, 066701 (2011)

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GERMICIDAL EFFICACY

GERMICIDAL EFFICACY

SENSITIVITIES AND WAVELENGTH DEPENDENCE

- UVGI = **u**ltra**v**iolet **g**ermicidal **i**rradiation
  - range: 200 – 300 nm
  - strongest effect: 260 - 270 nm, peak maximum at 265 nm
- killing of microorganisms is dose- and wavelength-dependent and differs between germs
- main target of UV radiation is DNA/RNA
- absorption of UV radiation different biomolecules
  - proteins < 240 nm
  - frequent amino acids: peaks at 220 nm, 280 nm

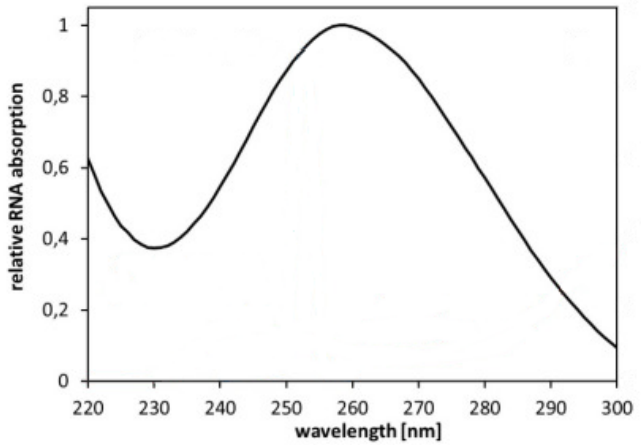

Fig. 9: relative UV absorption of RNA (modified after [3])

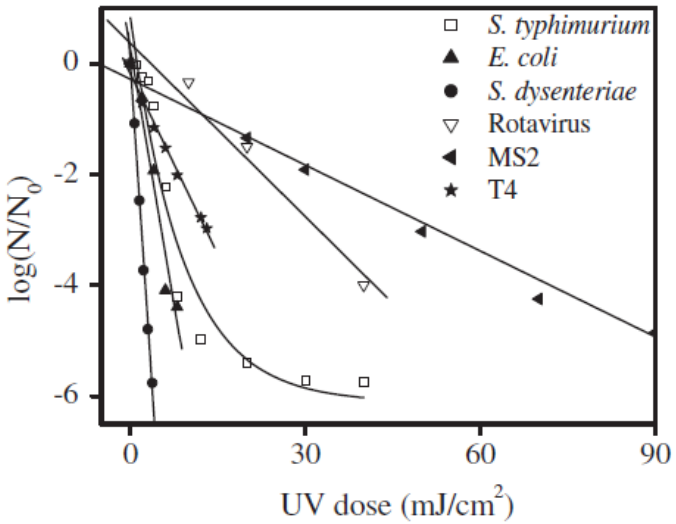

Fig. 10: Required UV radiation to kill selected microorganisms [4]

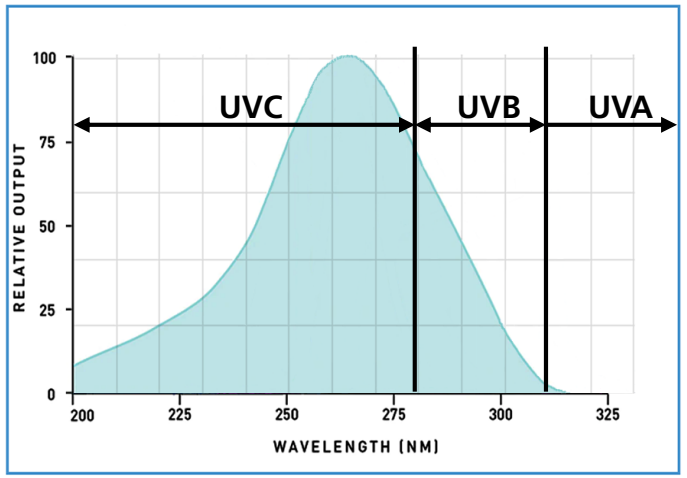

Fig. 8: UVGI effectiveness curve (modified after [URL-2])

[5]	Required fluence (mJ/cm²)			
MIC required (log):	1	2	3	4
Bacillus subtilis[a]	56	111	167	222
Adenovirus type 40	56	111	167	—[b]
Clostridium perfringens[a]	45	95	145	—[b]
Adenovirus type 2, 15, 40, 41	42	83	125	167
Acanthamoeba[c]	40	71	119	167
Adenovirus[a] (no type 40)	25	50	—[b]	—[b]
Calicivirus canine	10	21	31	41
Rotavirus SA-11	10	20	29	39
Calicivirus feline	9	19	28	38
Coxsackie virus B5	8	17	25	34
Streptococcus faecalis[a]	9	16	23	30
Legionella pneumophila[d]	8	15	23	30
Poliovirus type 1	7	15	22	30
Shigella sonnei[d]	6	13	19	26
Salmonella typhi[a]	6	12	17	51
Hepatitis A	6	11	17	22
Calicivirus bovine	5	11	16	21
E. coli O157[d]	5	9	14	19
E. coli[a]	5	9	14	18
Cryptosporidium USEPA[c]	3	6	12	—[e]
Giardia USEPA[c]	2	5	11	—[e]
Campylobacter jejuni[d]	3	7	10	14
Yersinia enterocolitica[d]	3	7	10	13
Legionella pneumophila[d]	3	6	8	11
Shigella dysenteriae[d]	3	5	8	11
Vibrio cholerae[d]	2	4	7	9

[URL-2] International Light Technologies, 2020, URL: https://www.intl-lighttech.com/sites/default/files/ilt2400_uvgi_response_2.png (last access 12.01.2022)
[3] Heßling et al., GMS Hygiene and Infection Control, Volume 15, Doc08 (2020) [4] Hu et al., Enviromental Engineering Science, Volume 29, 549-553 (2012)
[5] Hijnen et al., Water Research, Volume 40, 3-22 (2006)

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DAMAGE TO DNA/RNA (DIRECT)

- UVB and UVC photons damage the genome directly (bond breaks, photodimeric lesions)

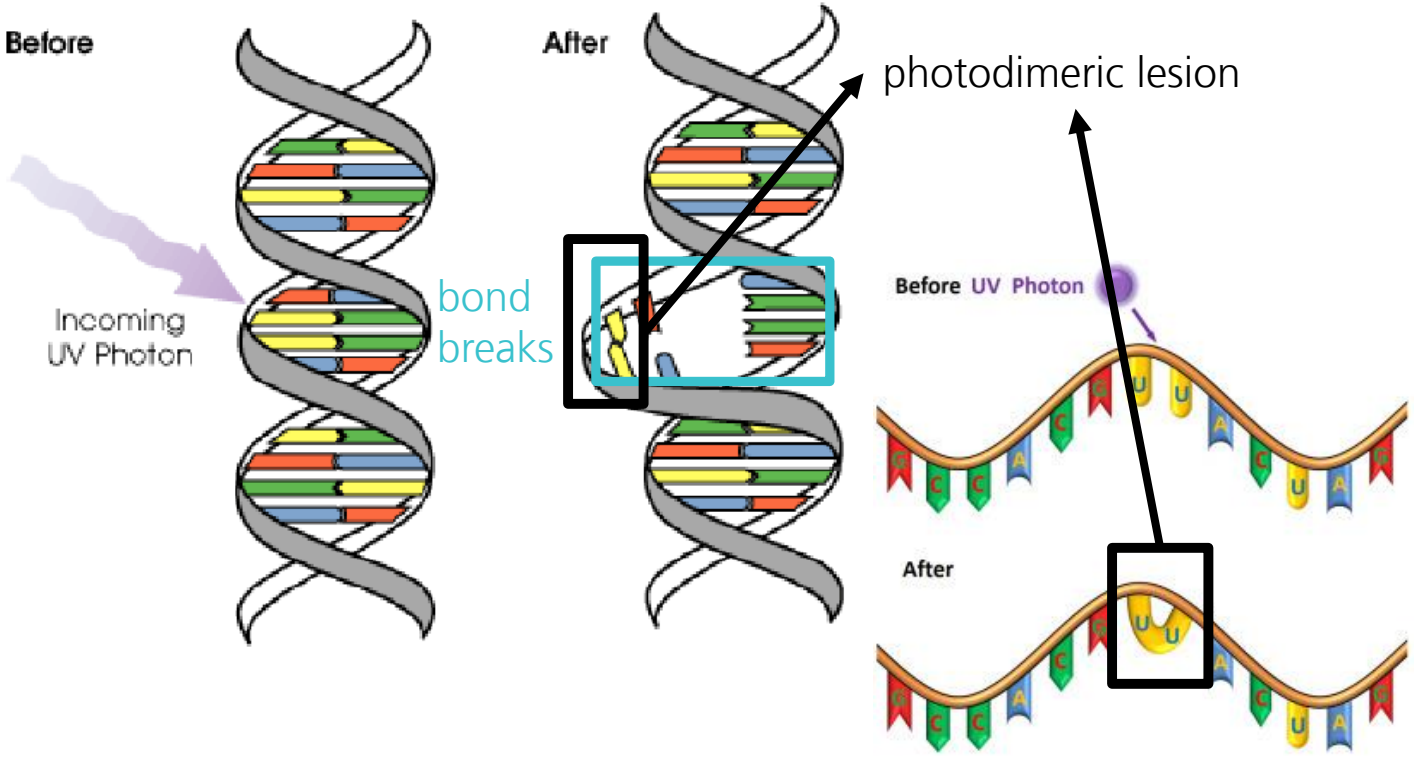

Fig. 12: Direct damage to DNA and RNA by UV radiation [3, 7]

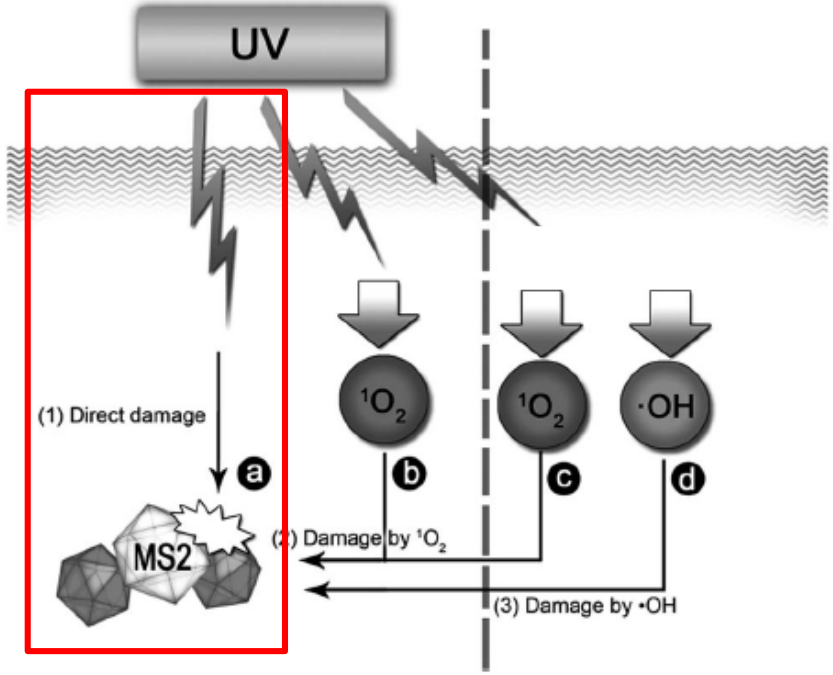

Fig. 11: UV inactivation mechanisms with the various processes of ROS generation [6]

[3] Heßling et al., GMS Hygiene and Infection Control, Volume 15, Doc08 (2020)
[6] Song et al., RSC Advances, Volume 5, 104779–104784 (2015)
[7] Mansoori et al., Nanotechnology in cancer prevention, detection and treatment, Volume 4, 226-257 (2007)

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GERMICIDAL EFFICACY

DAMAGE TO DNA/RNA (DIRECT)

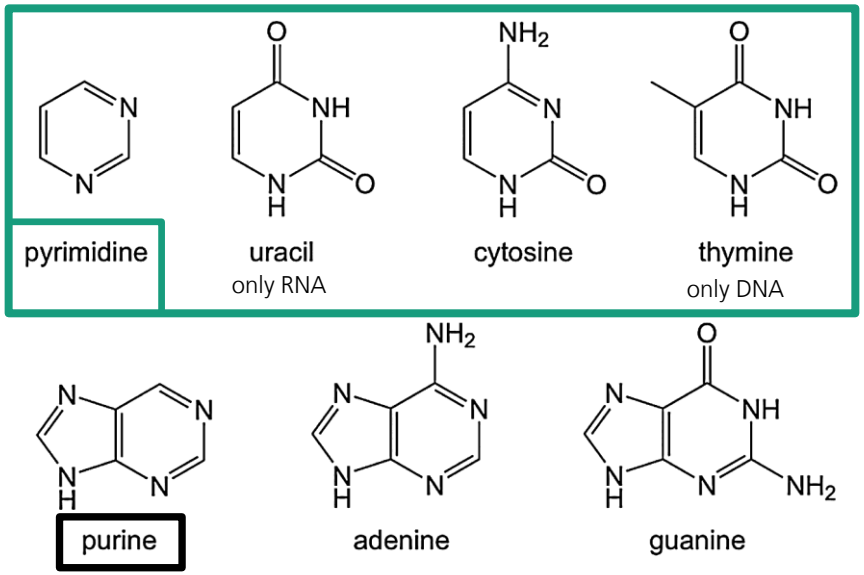


Fig. 13: Molecular structures of pyrimidine, purine and the nucleobases [8]

- main forms of UV-induced DNA damages:
  - cyclobutane pyrimidine dimers (CPDs)
  - pyrimidine-pyrimidone 6-4 photoproducts (6-4PPs)
  - Dewar valence isomer (only UVB)

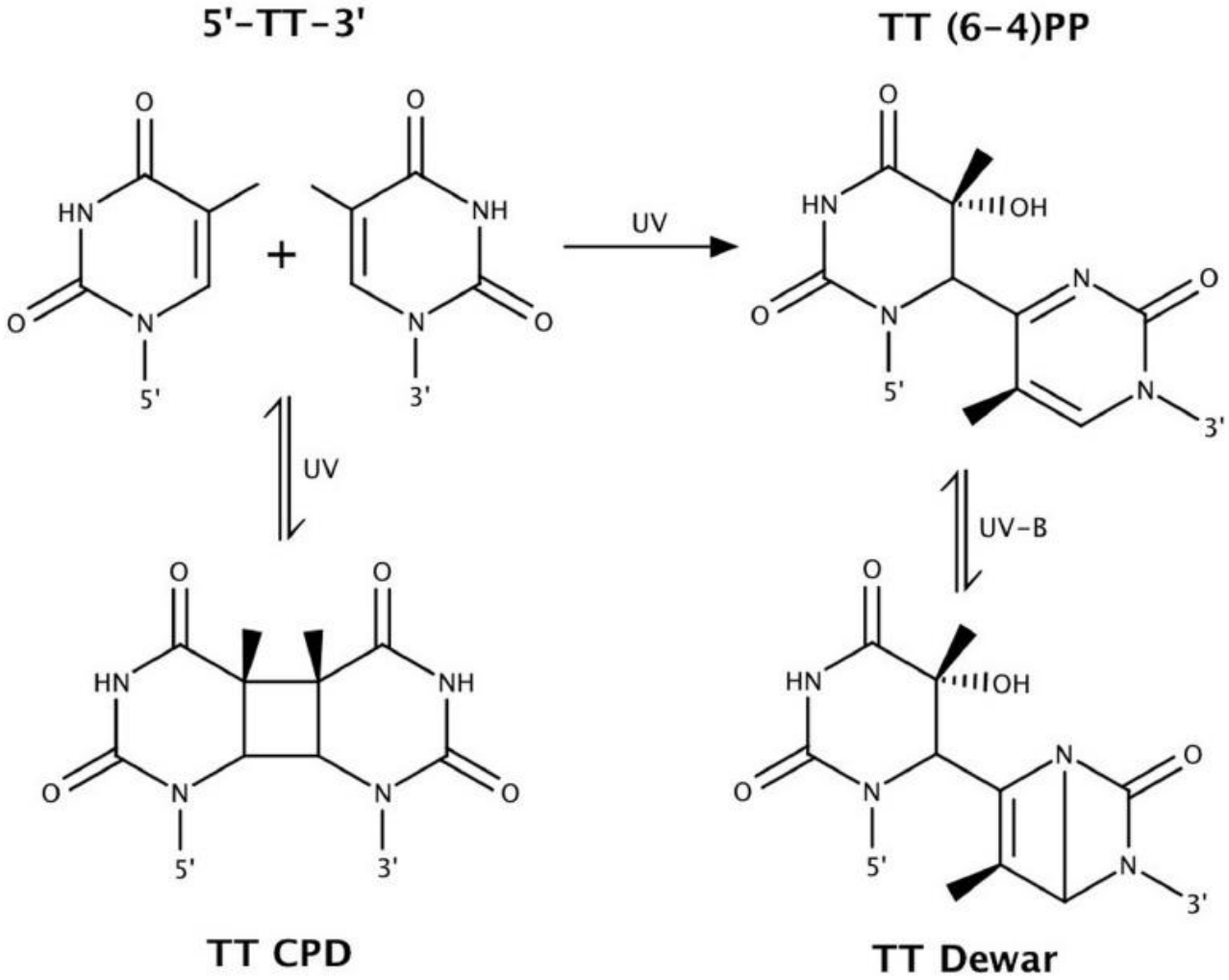


Fig. 14: Bipyrimidine lesions illustrated by the example of TT lesions [9]

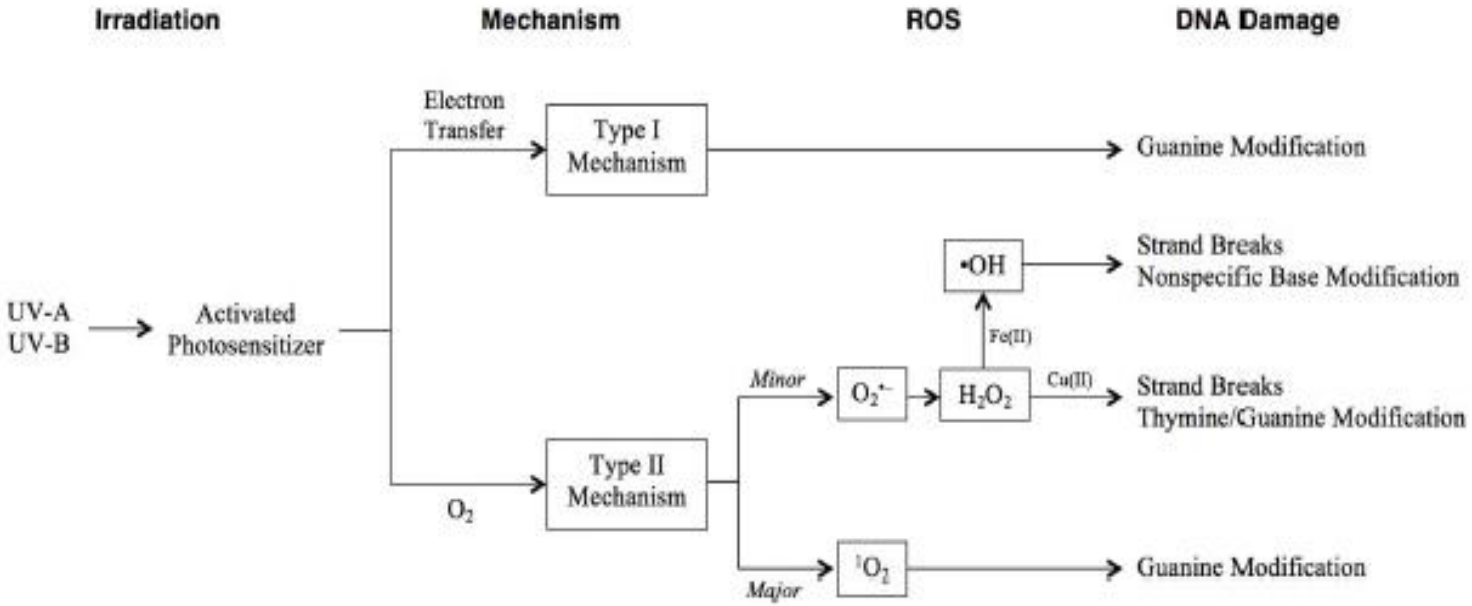
[8] Nuevo et al., RSC Astrobiology, Volume 9, 683-695 (2009)

[9] Jones and Baxter, Frontiers in Microbiology, Volume 8, 1882 (2017)

GERMICIDAL EFFICACY

DAMAGE VIA REACTIVE OXYGEN SPECIES (INDIRECT)

- reactive oxygen species (ROS) formation by absorption of UVA and UVB radiation
  → damage lipids, proteins and DNA
- photooxidative DNA damage (two types of mechanisms)
  - base modifications
  - strand breaks



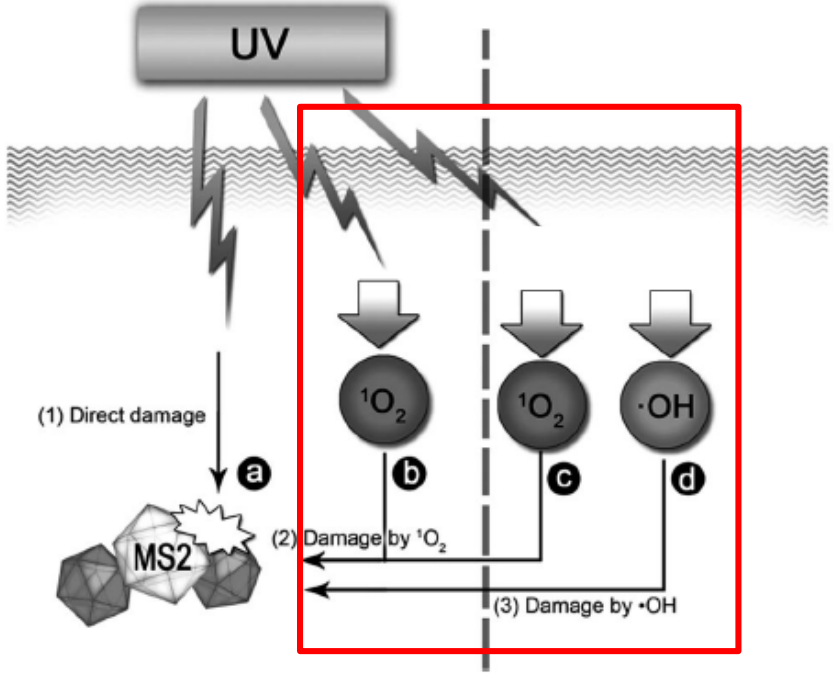


Fig. 11: UV inactivation mechanisms with the various processes of ROS generation [6]

oxidation potential:
guanine (1.40 eV) < adenine (1,75 eV) < thymine (2.00 eV) < cytosine (2.18 eV) < 2-deoxyribose

Fig. 15: Pathways of photooxidative DNA damage following UV irradiation [9]

[6] Song et al., RSC Advances, Volume 5, 104779–104784 (2015)
[9] Jones and Baxter, Frontiers in Microbiology, Volume 8, 1882 (2017)

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EFFECTIVENESS COMPARISON OF THE WAVELENGTH RANGES - AN EXAMPLE

- shorter wavelengths cause the greatest inactivation (UVB, UVC)
- killing through UVA radiation requires more time and a higher dose
- effect of UVB radiation is generally between the effectiveness of UVA and UVC

→ can activate the direct and indirect damage pathway

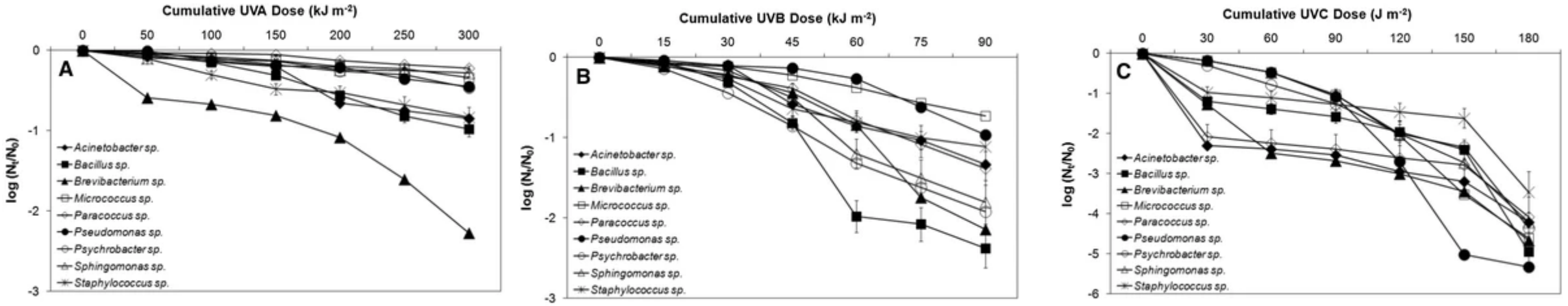

Fig. 16: UV sensitivity curves for some bacteria under the different UV spectral regions [10]

[10] Santos et al., Archives of Microbiology, Volume 195, 63-74 (2013)

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COMBINED APPLICATION WITH PHOTOCATALYSTS

- commonly used photocatalysts: TiO_2, ZnO, WO_3
- activation of two possible mechanisms through absorption of the energy of UV(A) radiation
  - **photocatalytic decomposition (photocatalysis)**
    - formation of ROS by redox reactions of electron-hole pairs with molecules adsorbed on the surface
    - possible ROS: **•OH, O2•- , •OOH, H_2O_2**
    - increase of ROS level (mainly **O2•-**) leads to oxidative stress and cell damage:
      - oxidation of proteins, amino acids, DNA
      - lipid peroxidation
  - **photoinduced (super)hydrophilicity**

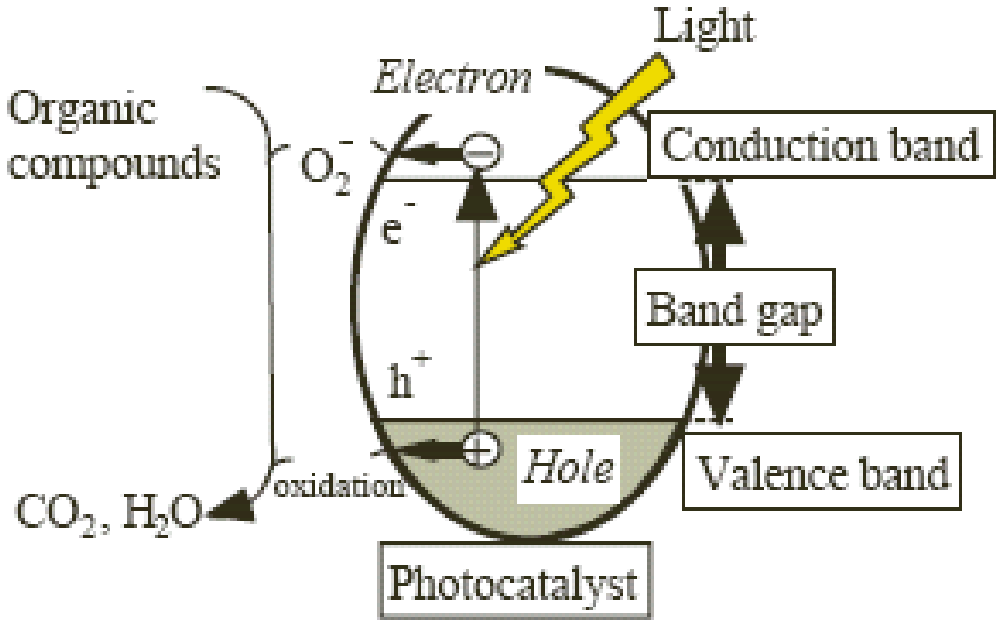

Fig. 17: Principle of photocatalysis [11]

[11] I. Abdel-Latiif, Nanocomposites - Recent Evolutions, IntechOpen (2018)

GERMICIDAL EFFICACY

COMBINED APPLICATION WITH PHOTOCATALYSTS

- commonly used photocatalysts: TiO_2, ZnO, WO_3
- activation of two possible mechanisms through absorption of the energy of UV(A) radiation
  - photocatalytic decomposition (photocatalysis)
  - **photoinduced (super)hydrophilicity**
    - extremely increased wetting behavior of aqueous solutions
    - disturbs or prevents germ adsorption and adhesion

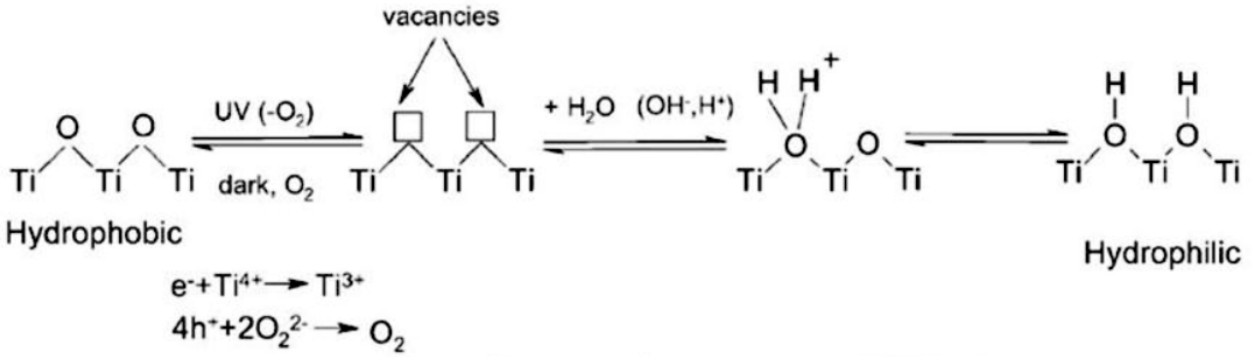

Fig. 18: Schematic representation of superhydrophilicity [12]

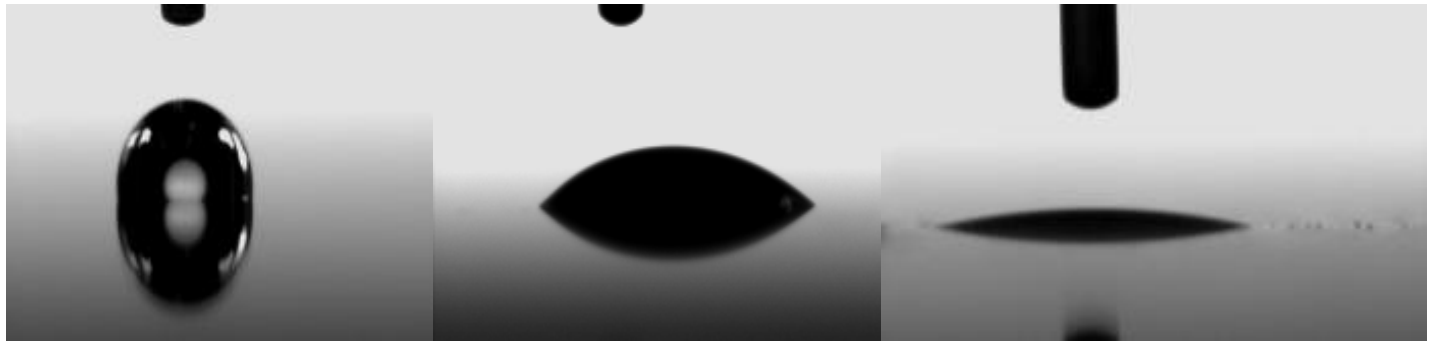

Fig. 19: Increase of the wetting behavior of a surface by UV irradiation

[12] J. Watté, Low Temperature Deposition of Photocatalytically Active TiO2 Coatings on Polymers, PhD thesis (2017)

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ANALYSIS OF DNA DAMAGE

methods for the detection of DNA damage

- antibody/fluorescence assay
- PCR/LAMP
- Bioluminescence
- comet assay
- radioactive labeling
- HPLC

example: antibody fluorescence assay

- fluorescent dye coupled with antibodies
- test can be used for the detection of CPDs

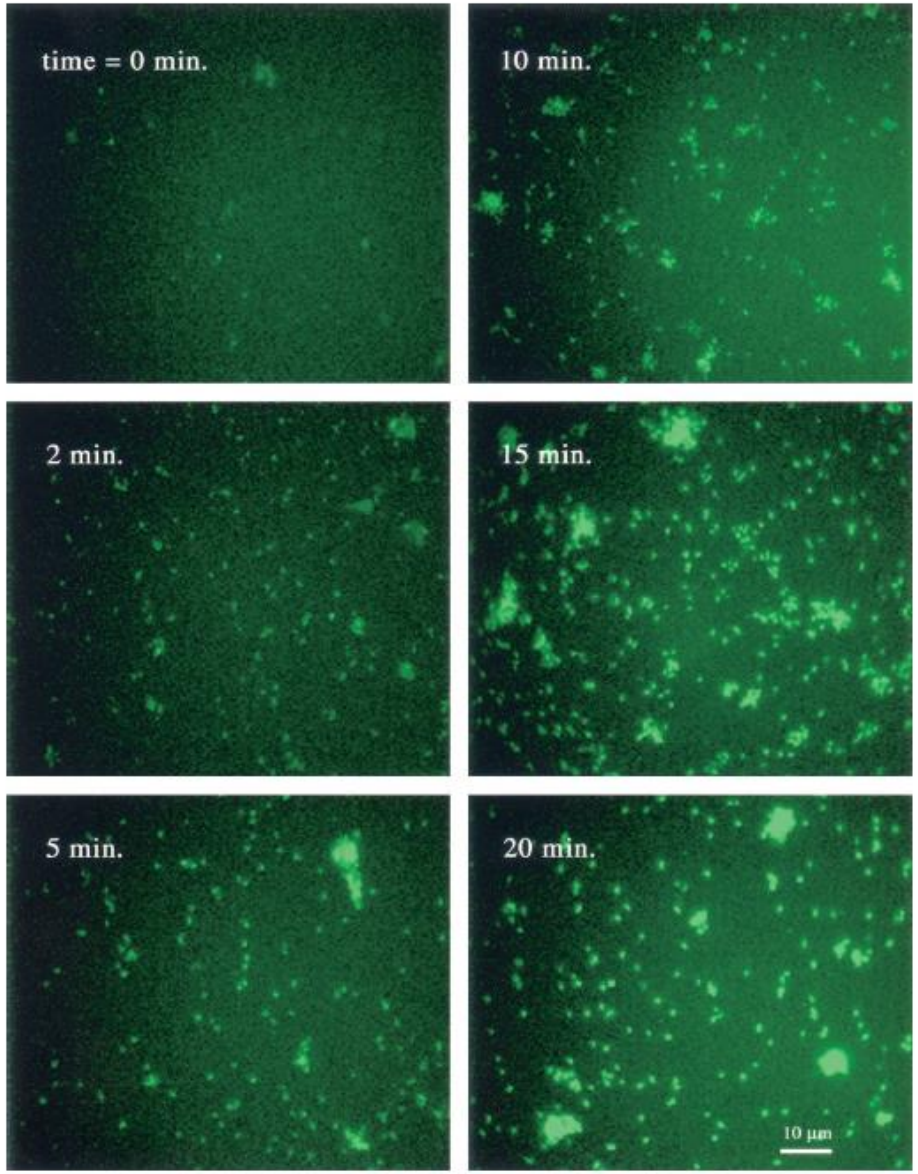

Fig. 20: Antibody fluorescence assay for the detection of CPDs after different UV irradiation times [13]

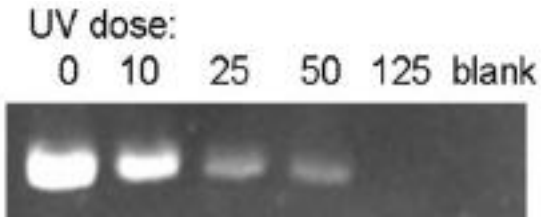

Fig. 21: PCR assay for relative DNA damage showing relative amplification [14]

example: PCR assay

- use of primers for the detection of undamaged DNA
- attenuation of bands by UV-induced DNA damage

[13] Peccia and Hernandez, Applied and Enviromental Microbiology, Volume 68, 2542-2549 (2002)
[14] Eischeid et al., Applied and Enviromental Microbiology, Volume 75, 23-28 (2008)

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REPAIR OF (PHOTO-INDUCED) DNA DAMAGE

REPAIR OF (PHOTO-INDUCED) DNA DAMAGE

- detection of the DNA lesion and initiation/execution of the repair by specialized repair proteins
- depending on the damage to the DNA, specific repair mechanisms are triggered

Repair mechanism	UV photodamage repaired [9]
Photoreactivation	Cyclobutane pyrimidine dimers, Pyrimidine (6-4) pyrimidone photoproducts Dewar isomers
Nucleotide excision repair (Sub-category: transcription-coupled repair)	Cyclobutane pyrimidine dimers Pyrimidine (6-4) pyrimidone photoproducts Dewar isomers
Base excision repair	Oxidative base lesions Single-strand breaks*

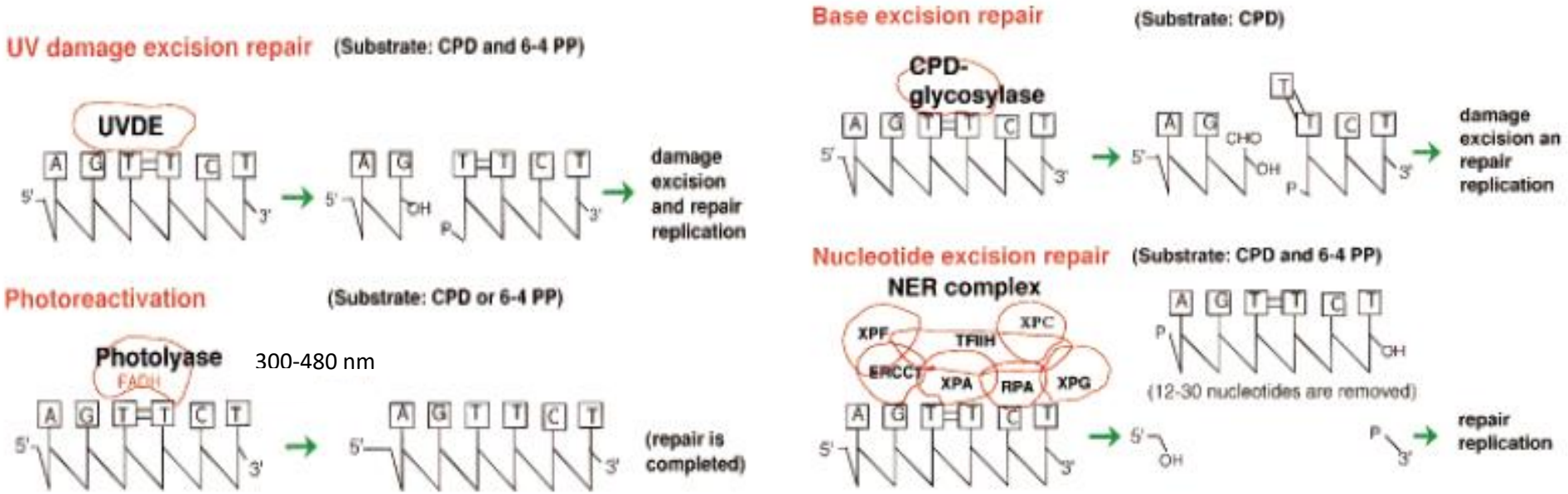

Fig. 22: Comparison of four repair pathways for UV induced DNA damage [15]

[9] Jones and Baxter, Frontiers in Microbiology, Volume 8, 1882 (2017)

[15] Yasui and McCready, BioAssays, Volume 20, 291-297 (1998)

REPAIR OF (PHOTO-INDUCED) DNA DAMAGE

PHOTOREACTIVATION

- radiation-dependent reaction
- repairs CPDs and 6-4PPs by splitting the dimer
- one photon can split about one dimer
- this mechanism has evolved early in evolution

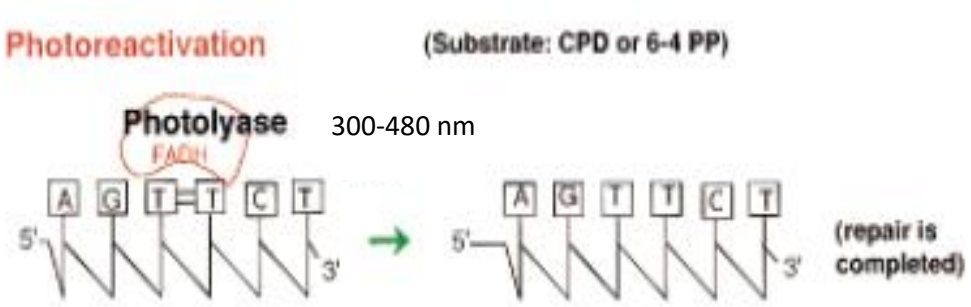

Fig. 23: Repair pathway (photoreactivation) for UV induced DNA damage [15]

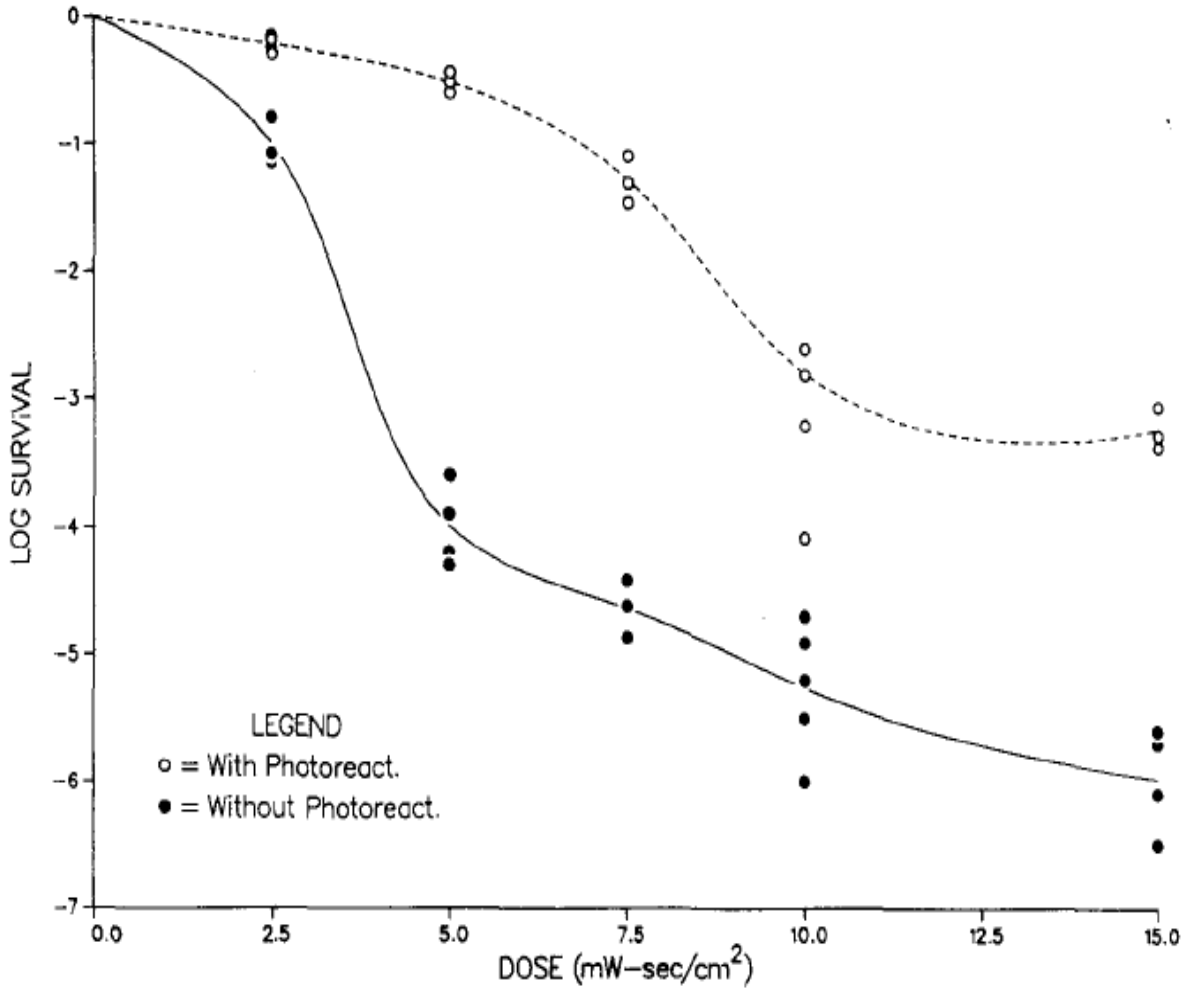

Fig. 24: Ultraviolet dose/log survival curves for *E. coli* with and without photoreactivation [16]

[15] Yasui and McCready, BioAssays, Volume 20, 291-297 (1998)

[16] Harris et al., Water Research, Volume 21, 687-692 (1987)

REPAIR OF (PHOTO-INDUCED) DNA DAMAGE

NUCLEOTIDE/BASE EXCISION REPAIR (NER, BER)

- dimer is excised by cellular enzymes and replaced by a monomer

NER

- primary mechanism for removal of UV-induced cellular damage
- a protein complex recognizes, cut and remove the damaged DNA
- new DNA strand is synthesized

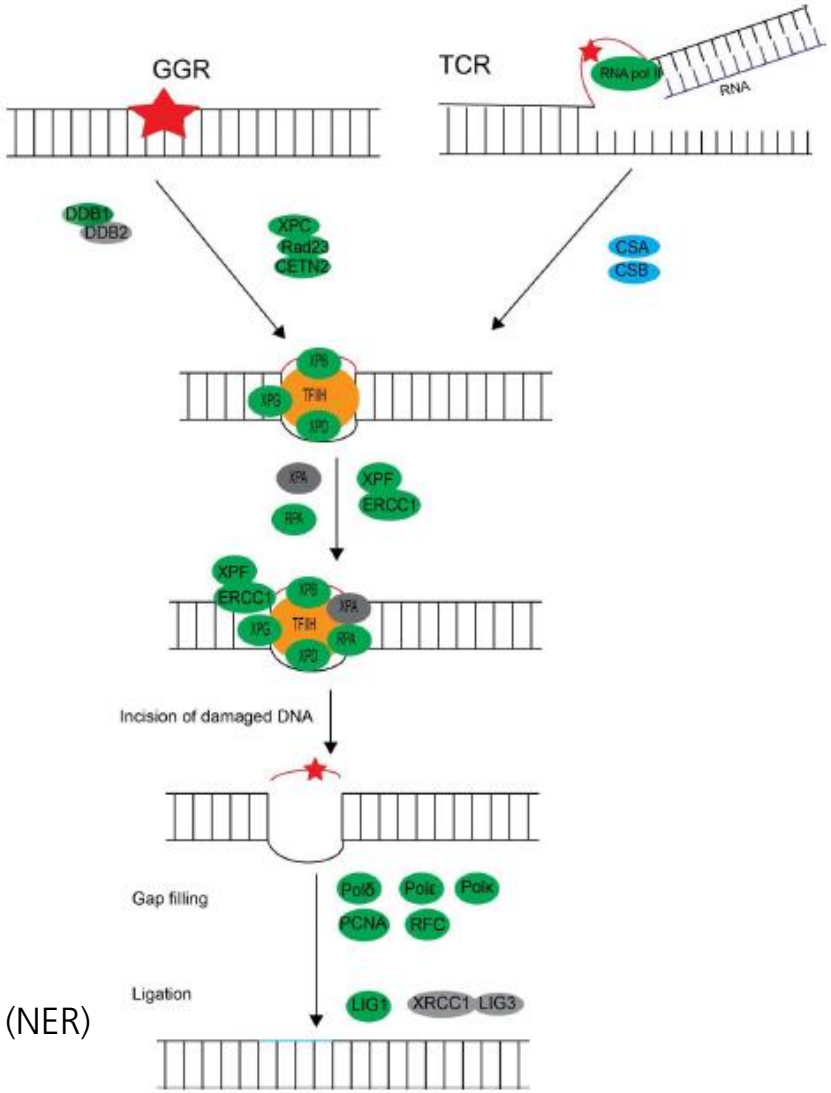

Fig. 25: Nucleotide excision repair (NER) pathway in dinoflagellates [17]

[17] Li and Wong, Microorganisms, Volume 7, 191 (2019)

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REPAIR OF (PHOTO-INDUCED) DNA DAMAGE

NUCLEOTIDE/BASE EXCISION REPAIR (NER, BER)

- dimer is excised by cellular enzymes and replaced by a monomer

BER

- mainly repairs damage by ROS or direct ionizing radiation
- removes damaged or altered bases in DNA
- bond between the base and the deoxyribose ring is cleaved and new nucleotides(s) are inserted

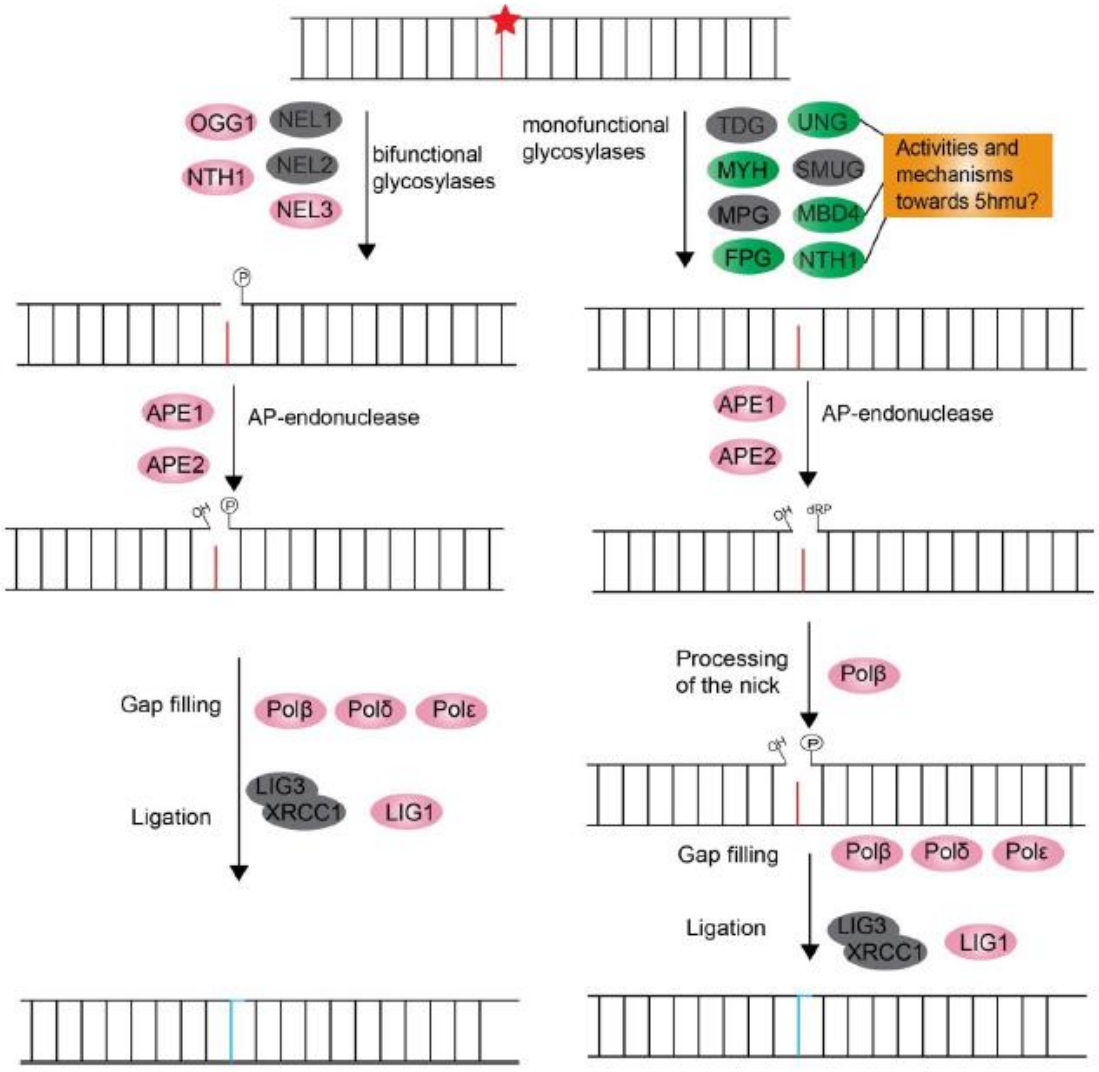

Fig. 26: Base excision repair (BER) pathway in dinoflagellates [17]

[17] Li and Wong, Microorganisms, Volume 7, 191 (2019)

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REPAIR OF (PHOTO-INDUCED) DNA DAMAGE

VBNC STATUS ANALYSIS

- VBNC = viable but not countable

methods for the detection of the VBNC status

- PMA-PCR
- direct viable count (DVC)
- live/dead systems
- detection of metabolic activity: ATP assay
- detection of enzymatic activity: esterase/ dehydrogenase activity
- protein synthesis: DAPI vs. FISH

example: ATP assay

- adenosine triphosphate (ATP) = nucleotide and universal energy carrier in cells

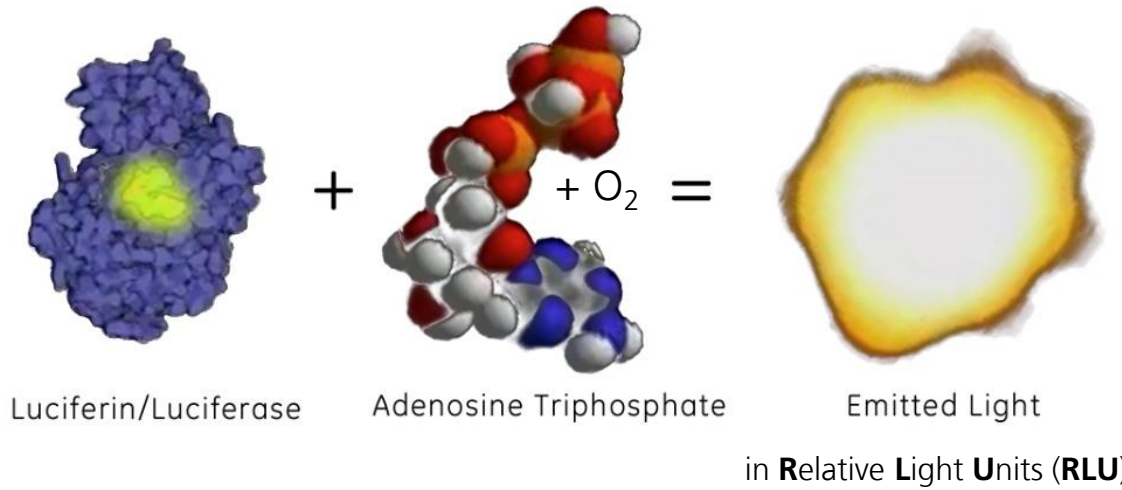

Fig. 27: Detection reaction of the ATP test [URL-3]

[URL-3] Hygiena LLC, 2022, URL: https://www.hygiena.com/frequently-asked-questions/ ((last access 08.03.2022)

REPAIR OF (PHOTO-INDUCED) DNA DAMAGE

VBNC STATUS ANALYSIS

example: immunofluorescence assay

- green: damaged bacteria (CPDs)
- blue: all bacteria (DNA)
- PER = photoenzymatic repair → photoreactivation

→ detection of photoreactivation by reduced number of green fluorescent bacteria

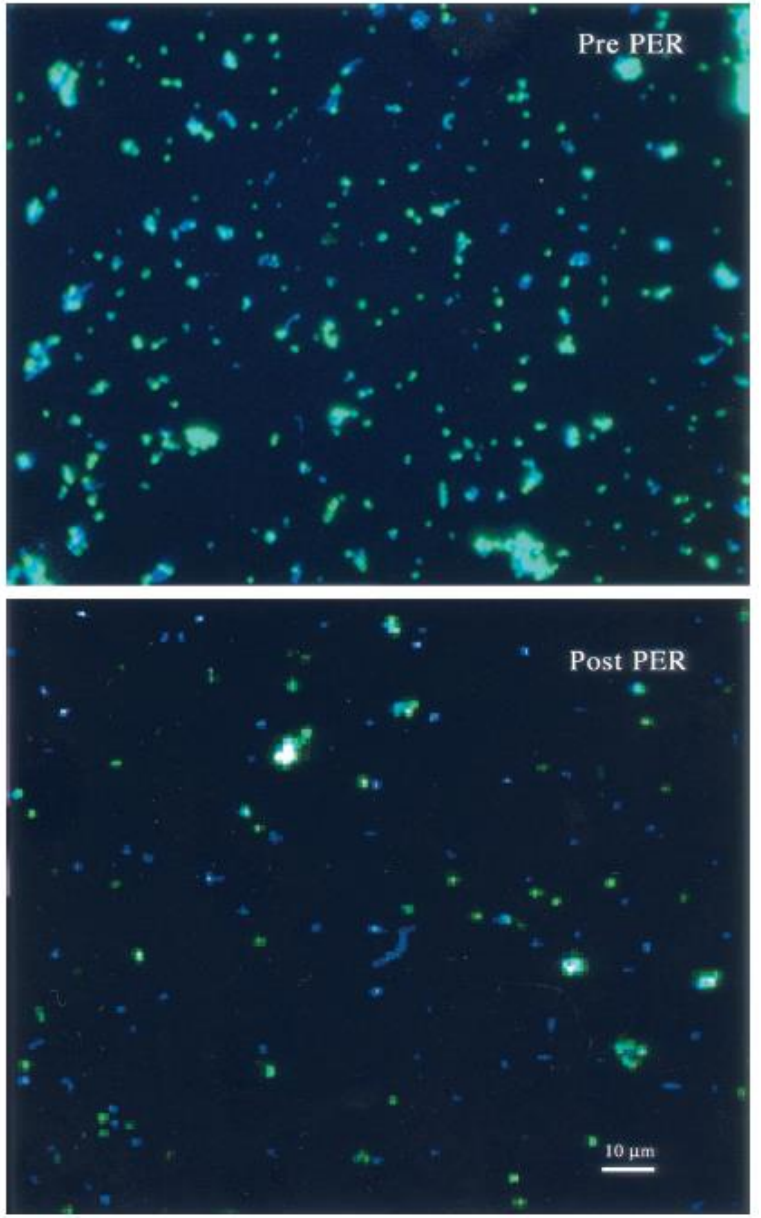

Fig. 28: Fluorescence-labeled bacterial suspensions before and after photoreactivation experiments [13]

[13] Peccia and Hernandez, Applied and Enviromental Microbiology, Volume 68, 2542-2549 (2002)

COMPARISON WITH OTHER DISINFECTION AND STERILIZATION METHODS

COMPARISON WITH OTHER DISINFECTION AND STERILIZATION METHODS

disinfection/sterilization methods:

chemical disinfection, filtration, annealing & flaming, gases, gamma radiation, hot air sterilization, antimicrobial agents, autoclaving, pasteurization, plasma etc.

Advantages and disadvantages of UV treatment

+	simple method without additional agents	-	harmful to eyes, skin and genetic material of humans, as well as materials → protective gear necessary
+	broadband effect (viruses, bacteria, fungi, multi-resistant germs)	-	wavelengths < 240 nm: ozone production
+	effective and efficient	-	mercury tubes = risk of pollution
+	can be used over large areas	-	cost and availability of UVC sources
+	no dangerous gas emissions or wastes	-	air (clinical area) and water (water treatment): effective only with sufficiently long contact time
+	relatively low operating costs	-	low penetration depths (pores, dirt etc.)
+	more efficient than manual cleaning with disinfectants	-	treatment times depending on the germ
+	short treatment times for surfaces	-	risk of resistance formation and mutation

GENERATION OF ARTIFICIAL UV RADIATION - ADVANTAGES AND DISADVANTAGES OF LEDS

GENERATION OF ARTIFICIAL UV RADIATION - ADVANTAGES AND DISADVANTAGES OF LEDS

+ narrow band (monochromatic) and high intensity
+ specific adaptation to application possible
+ stable spectral output power at a given temperature
+ almost unlimited number of switching cycles
+ provide full light output immediately (no warm-up time required)
+ high efficiency
+ lenses allow focus on specific area
  → increased radiant power per m^2
+ for applications where sources have to be as small as possible
+ less harmful to the environment

- smaller spectrum than gas vapor lamps (227-400 nm)
- cost factor
- (only single wavelengths possible) → LED arrays
- (only small areas can be irradiated per LED)
  → LED arrays

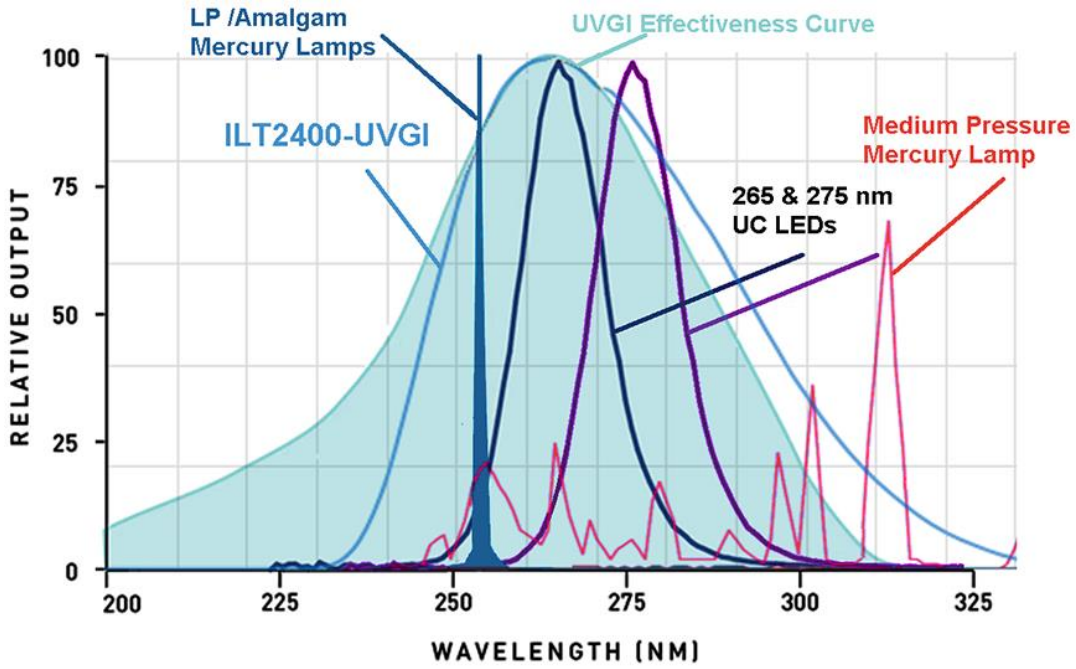

Fig. 29:Comparison of different UV lamp spectra with UV sensitivity of microorganisms [URL-2]

[URL-2] International Light Technologies, 2020, URL: https://www.intl-lighttech.com/sites/default/files/ilt2400_uvgi_response_2.png (last access 12.01.2022)

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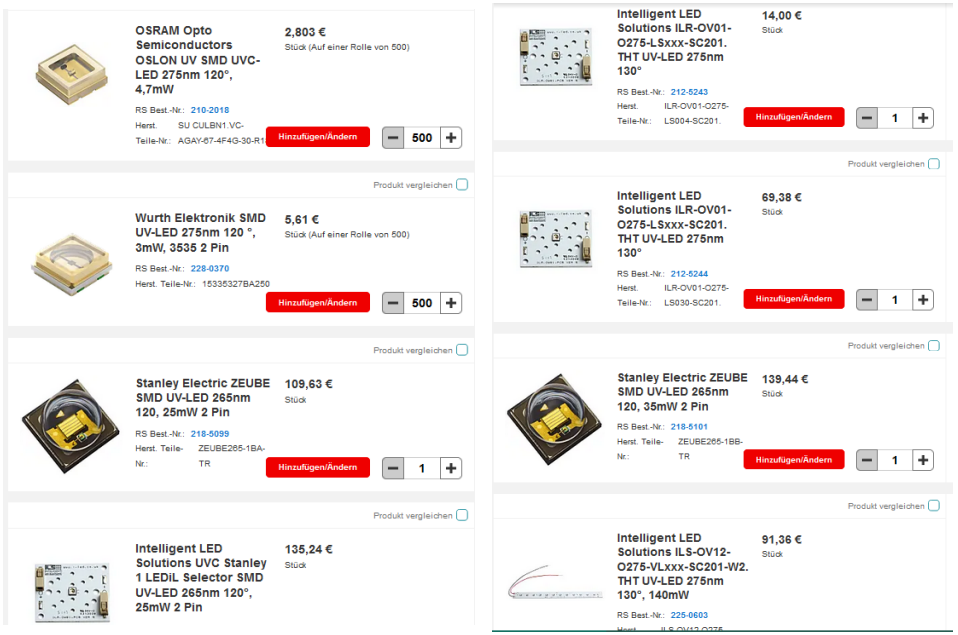
CURRENT RESEARCH, PROJECTS AND PRODUCTS

CURRENT PRODUCTS, PROJECTS AND RESEARCH

CURRENT MARKET SITUATION/PRODUCTS

- many cost-effective products for disinfection/sterilization
- costs for the products do not match the prices for UV-LEDs
  → often use of blue LEDs
- blue LEDs require much higher doses for disinfection than UVC LEDs (factor 10,000)
- **Caution**: blue LEDs can activate photoreactivation and are harmful to the eye



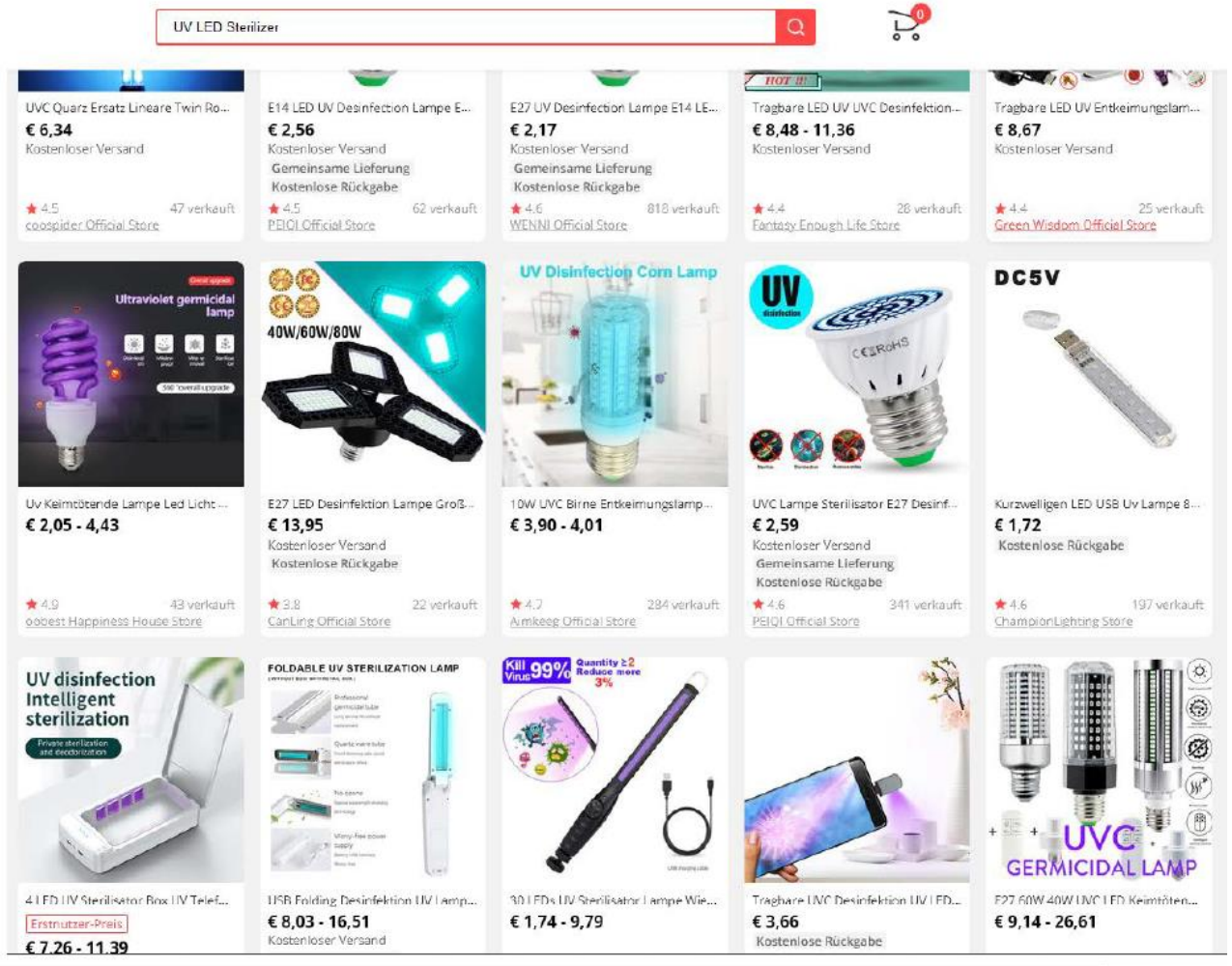


Fig. 30 & 31:UVC-LEDs of common manufacturers (left) and various UVC-LED products for sterilization and disinfection (according to the manufacturer; top)

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CURRENT PRODUCTS, PROJECTS AND RESEARCH

PROJEKTS AT FRAUNHOFER FEP

- Fraunhofer Anti-Corona Projekt „**Mob**ile **Di**sinfection (MobDi)“
- 12 Fraunhofer Institutes developed new robotic solutions for autonomous efficient and gentle cleaning and disinfection of surfaces in various application environments
- Fraunhofer FEP: evaluated the disinfection success of the tools developed, which included a UV LED emitter

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Fig. 32 & 33: UV LED emitter (top) and autonomous disinfection robot equipped with the source (right)

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This work was supported by the Fraunhofer Internal Programs under Grant No. Anti-Corona 840264.

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CURRENT PRODUCTS, PROJECTS AND RESEARCH

PROJEKTS AT FRAUNHOFER FEP

Gefördert durch:

Bundesministerium für Wirtschaft und Energie

aufgrund eines Beschlusses des Deutschen Bundestages

- **problem**: siphons = high risk of infections in hospitals
- **solution**: modification of a siphon with a photocatalytic active titanium dioxide coating and UV-LEDs
- superhydrophilicity is stable for several months
- the stable superhydrophilic effect results in the complete prevention of germ adhesion and accumulation on curved geometries

In cooperation with:

MOVEOMED

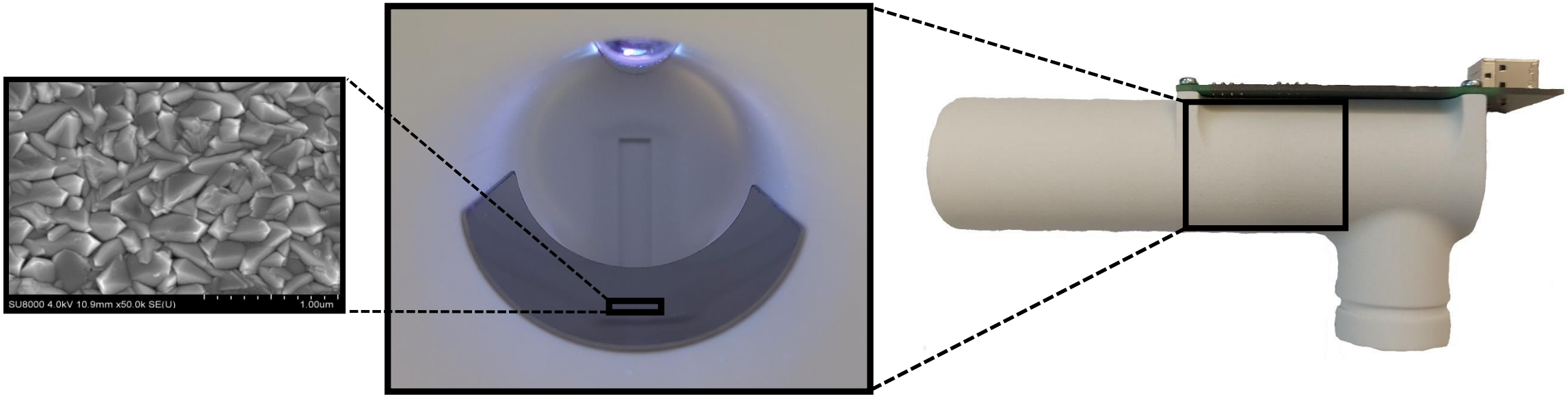

Fig. 34: Functionalized siphon prototype, which is equipped with a UVA-LED (365 nm) and a titanium dioxide coated specimen

The project was funded by the Federal Ministry for Economic Affairs and Energy (ZF4597702BA8).

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CURRENT PRODUCTS, PROJECTS AND RESEARCH

RESEARCH IN FAR UVC

- far UVC: 207-222 nm
- studies often performed on KrCl excimer lamps (222 nm)

statements made so far about far UVC:

- inactivation of pathogenic viruses is at least, and often more effective as compared with conventional UVC
- no evidence, to date, of any adverse human eye or skin damage
- sources with significant emission above 230 nm may require optical filtering
- ozone generation has been observed and should be considered and measured during application design

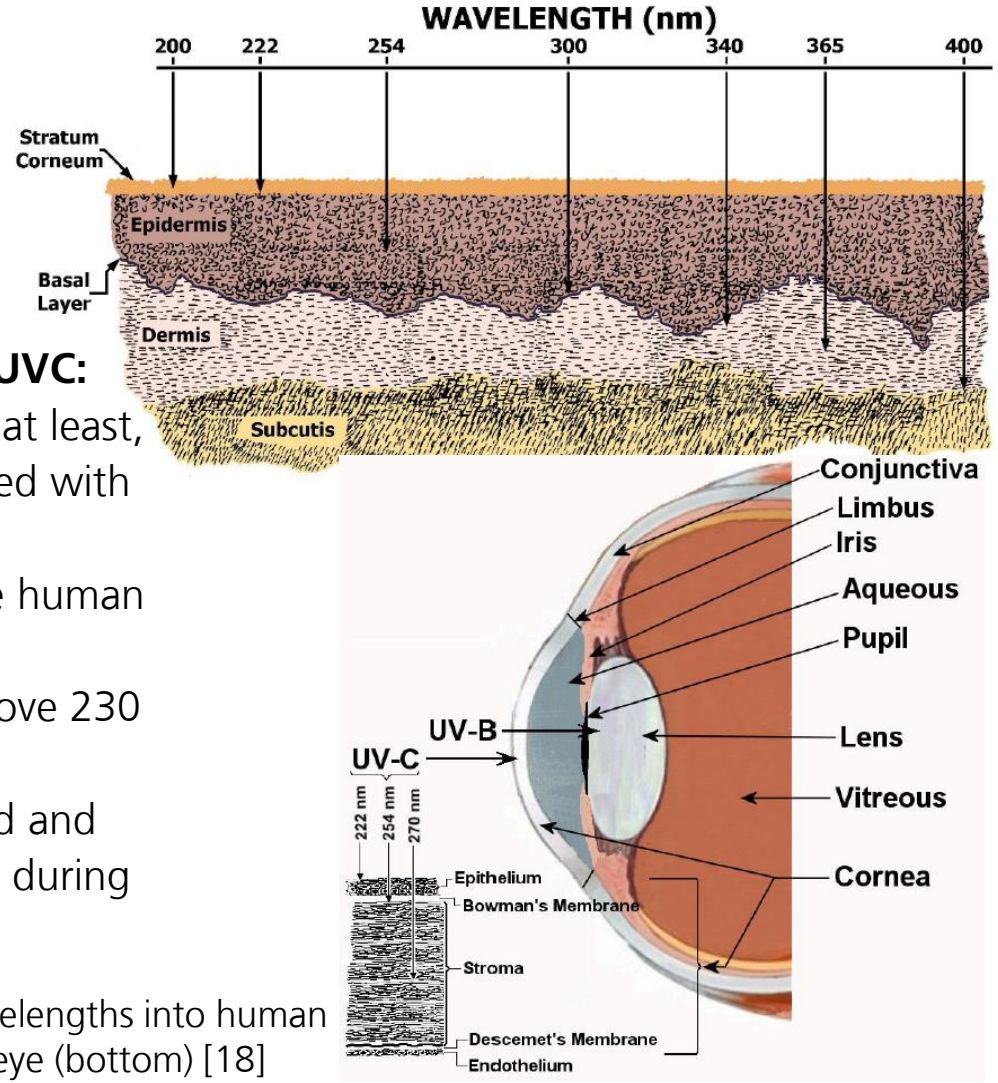

Fig. 34: Penetration of UV wavelengths into human skin (top) and into the human eye (bottom) [18]

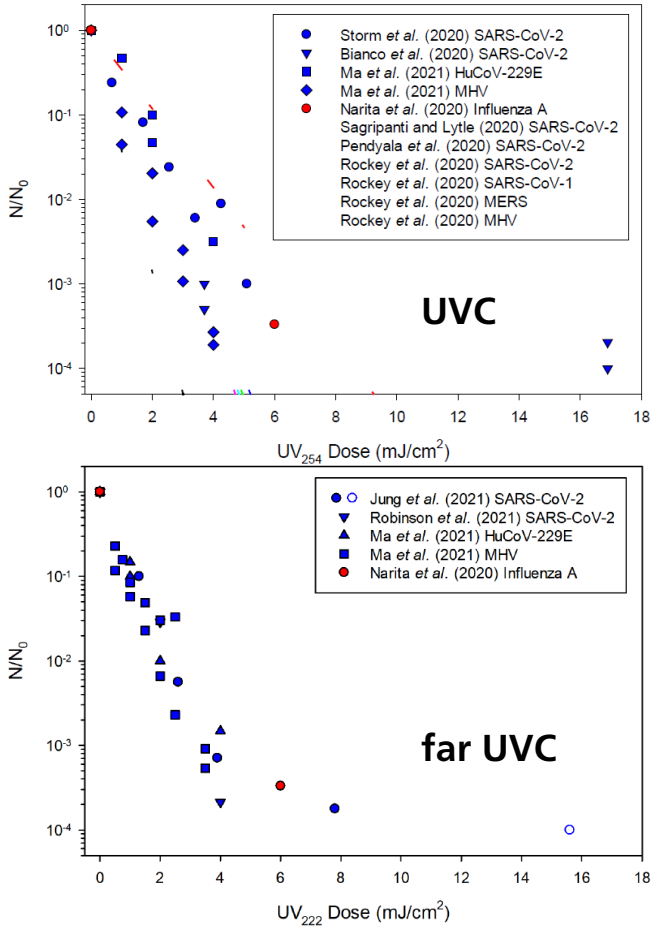

Fig. 35: Measured dose-response behavior of common respiratory ssRNA viruses in aqueous suspension [18]

[18] Blatchley III et al., Far UV-C Radiation: Current State-of Knowledge, IUVA Task Force (TF) on Far UV-C Radiation for Disinfection of Air and Surfaces, White Paper (2021)

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SUMMARY

SUMMARY

- increased use of UV radiation, already established in water and air disinfection, food sterilization, surface decontamination and disinfection of medical equipment

- UVGI: 200 – 300 nm
- UVC directly damage genetic material, UVA damages through ROS formation, UVB uses both damage pathways
- combination of UV radiation with photocatalysts: triggering of superhydrophilicity and/or photocatalysis

- photo-induced DNA damage ca be bypassed by specific repair mechanisms (photoreactivation, BER, NER)

- UV radiation has many advantages compared to other disinfection and sterilization methods, but also two big disadvantages:
  - possible damage to humans (without protection)
  - high costs (UV LEDs)

forecast: due to the rapid progress in research, disinfection with UV LEDs will expand the fields of application and use of radiation in the coming years

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Thank you for your attention!

Questions?

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