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What happens during "Sodis"?

A deeper insight into cellular damages caused by sunlight

Franziska Bosshard
Michael Berney
Margarete Bucheli-Witschel
Hans-Ulrich Weilenmann
and Thomas Egli

Girona, October7-8, 2009

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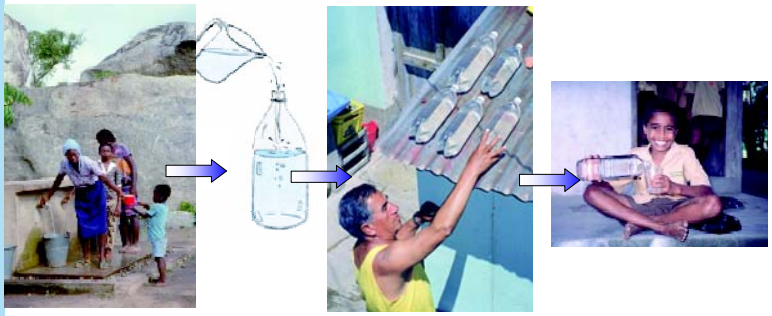
Solar disinfection

SODIS Solar Water Disinfection

Source water → Fill into PET bottle → Expose to sun (> 5h) → Drink



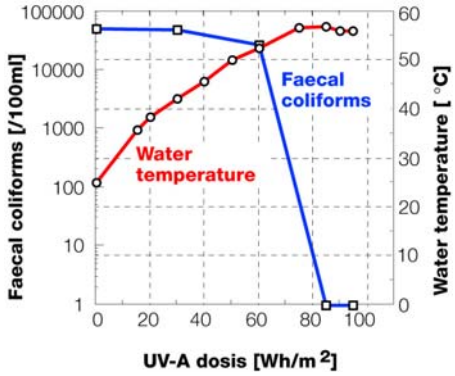
2

Experiments show inactivation

3

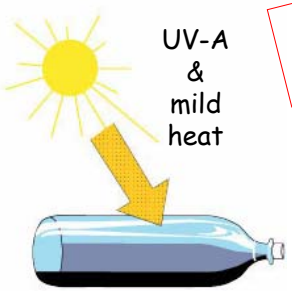
Exposure in PET bottles shows:

- *water may heat up to more than 50 °C*
- *faecal coliforms are reduced by 3-4 logs*

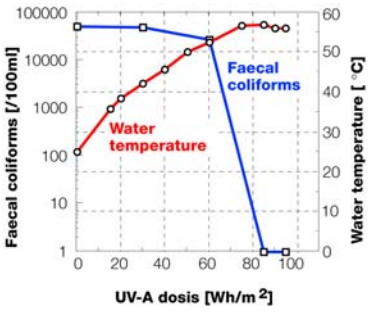


Promoted by WHO since 2004...

4



... as a simple, cheap and safe technique



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Introduction and overview light exposure systems

Importance of cellular state

Multi-parameter assessment of microbial viability and death

Membrane proteins as primary targets

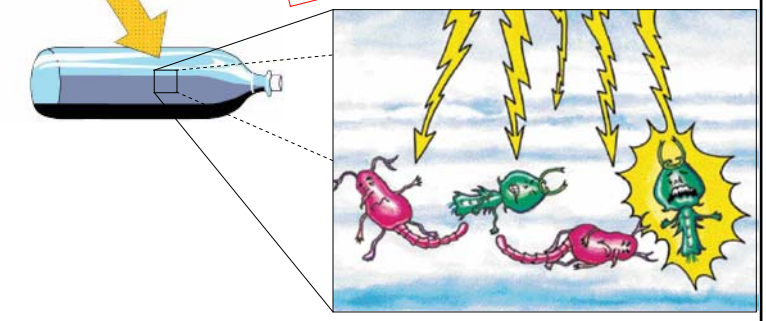
Take-home messages

5

Promoted by WHO, but frequently consumers and promoters ask:

UV-A & mild heat

"This is too simple, this cannot be safe !!! What is the inactivation mechanism for pathogens?"



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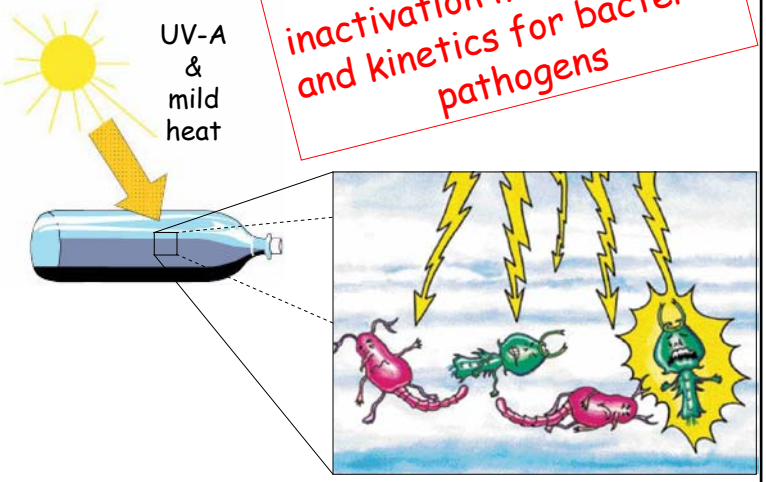
6

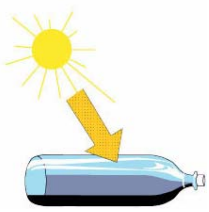
Our goal:

UV-A & mild heat

Elucidate the main inactivation mechanism(s) and kinetics for bacterial pathogens



Experimental systems used for exposure



Laboratory

- "merry-go-round" reactor
- continuous culture reactor

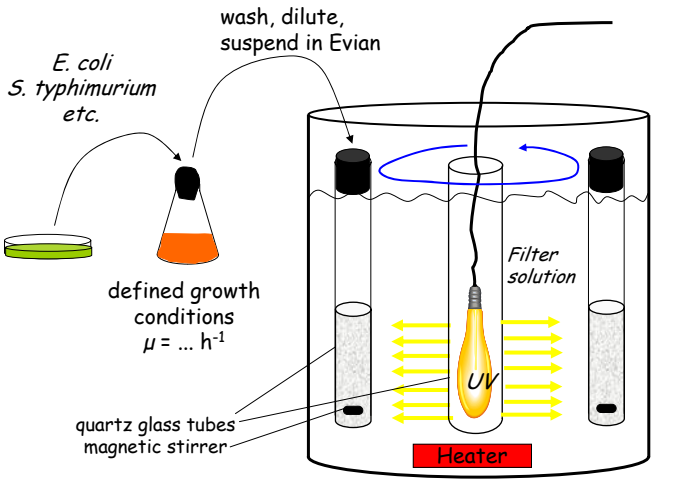
(T, intensity, wavelength etc. control)

Field reactor

(T, wavelength control)

Field experiments with bottles

Laboratory: „artificial sunlight" in the „merry-go-round reactor"



Filter solution KNO_3, cut-off 320nm, halfmax 340nm; fluence rate determined also by actinometry

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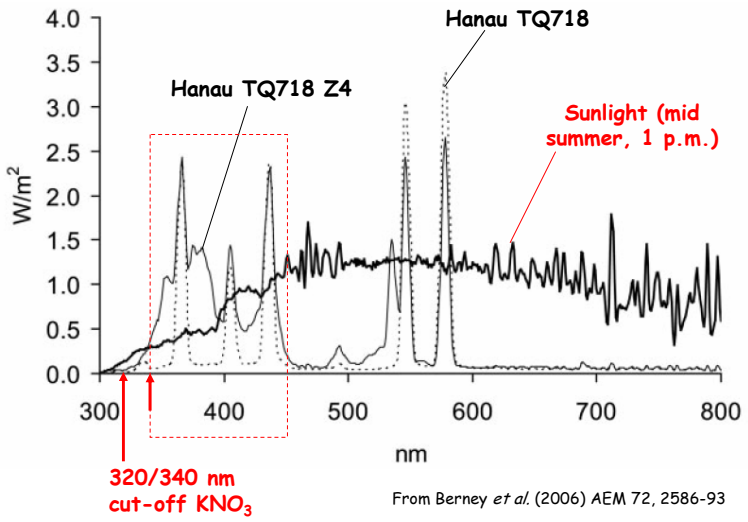
Take-home messages

9

Spectra of used lamps

(corrected for filter solution $NaNO_3$)



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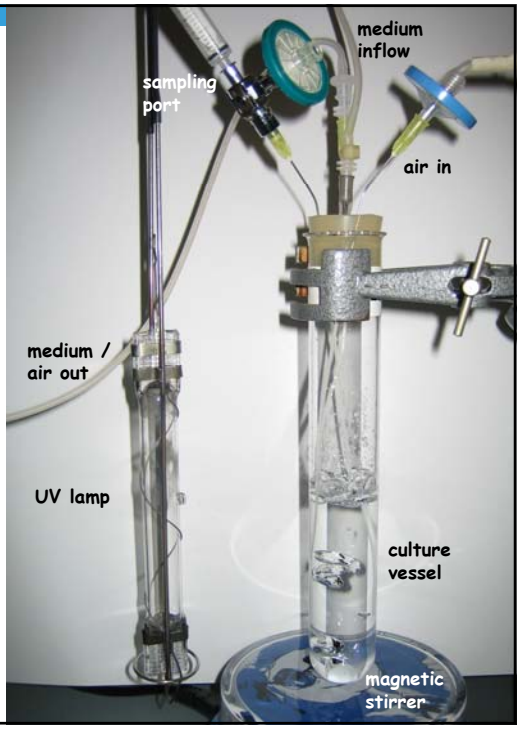
Take-home messages

10

Low volume, simple continuous culture

for exposure of cells growing at defined rate in "merry-go-round" reactor



Field reactor: exposure to sunlight

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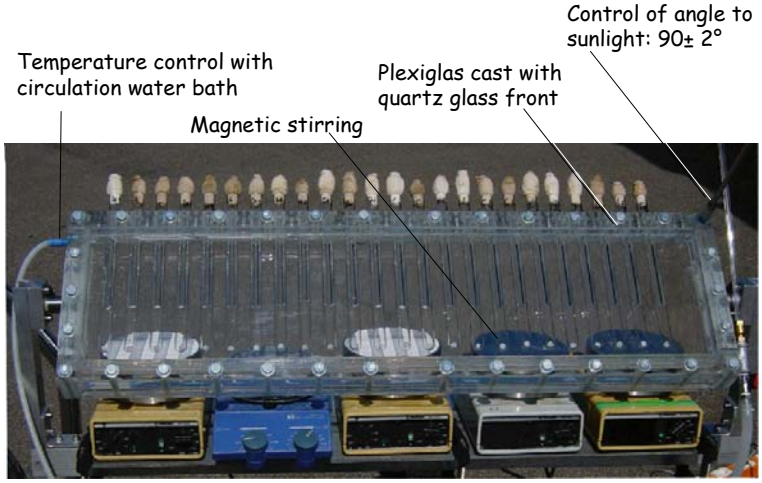
- Take-home messages



Daily sun irradiation data from BUWAL/NABEL weather station 300m away at EMPA Dübendorf, fluence calculated for 350-450nm range

11

Average sunlight intensities in Dübendorf

- Introduction and overview light exposure systems
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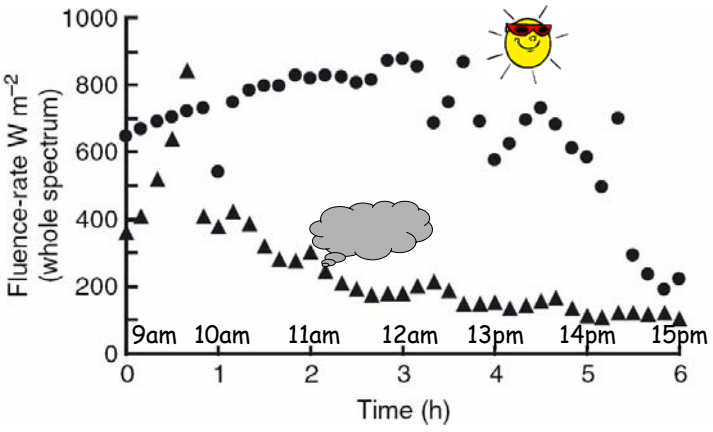

Data from BUWAL/NADUF station, Dübendorf (for range 350-450nm)

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12

Introduction and overview light exposure systems

Importance of cellular state

Multi-parameter assessment of microbial viability and death

Membrane proteins as primary targets

Take-home messages

13

Today's focus

1) Importance of pre-cultivation

2) Importance of using several parameters simultaneously for assessment of cell viability

3) Use of flow cytometry-based methods in combination with conventional methods

4) Damage induced by (artificial) solar radiation

Introduction and overview light exposure systems

Importance of cellular state

Multi-parameter assessment of microbial viability and death

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Take-home messages

14

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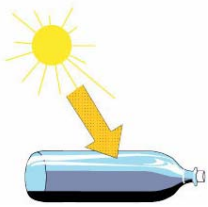

1) Importance of pre-cultivation

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Indications in the literature:

microbial resistance to disinfectants and stress can depend on growth rate

Increased stress resistance during slow growth for ClO_2

Survival of *Legionella pneumophila* and *E. coli* after exposure to 0.75 mg/L ClO_2 for 10min (dose = 7.5 mg · min · L^{-1})

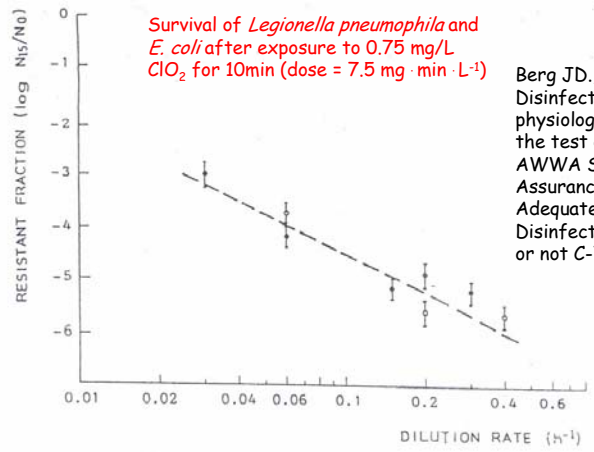


Figure 5 Effect of dilution rate on sensitivity to a dose of 0.75 mg/l ClO_2. L. pneumphila (•); E. coli (○). Growth temperature was 35°C and nutrient-limited for both species. (Berg et al., 1985).

Berg JD., 1987. Disinfection: The physiological state of the test organism. In: AWWA Seminar on Assurance of Adequate Disinfection, or C-T or not C-T, pp.85-99.

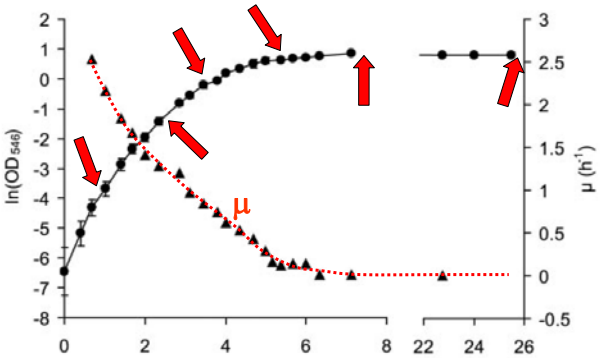
SODIS literature: What cells were used in disinfection experiments?

17

Frequently described in M&M:

"For disinfection experiments a culture of *E. coli* was grown in LB and harvested in the ..... phase":

- early exponential
- early stationary
- exponential
- stationary
- late exponential
- late stationary...



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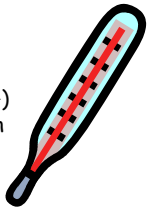
Increased resistance during slow growth to SODIS stresses

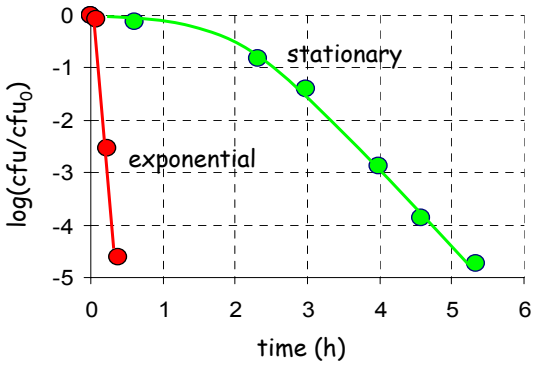
Introduction and overview light exposure systems
Importance of cellular state
Multi-parameter assessment of microbial viability and death
Membrane proteins as primary targets
Take-home messages

18

E. coli MG1655:
Grown in LB, batch, harvested in exponential phase ($\mu=2h^{-1}$) or stationary phase ($\mu=0h^{-1}$), washed 2x, suspended in Evian



48°C



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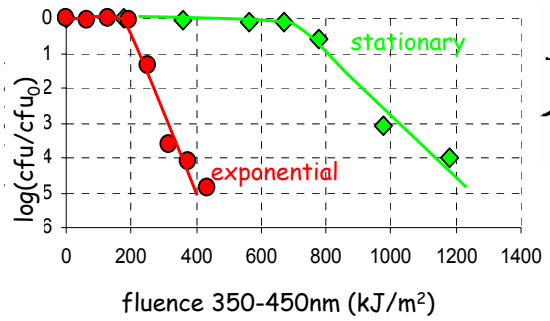
Increased resistance during slow growth to SODIS stresses

E. coli MG1655:
Grown in LB, batch, harvested in exponential phase (μ = 2 h^{-1}) or stationary phase (μ = 0 h^{-1}), washed 2x, suspended in Evian and exposed at 20°C to sunlight



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Inactivation patterns: Shoulder (log-linear), no shoulder (linear), or biphasic linear?

Introduction and overview light exposure systems
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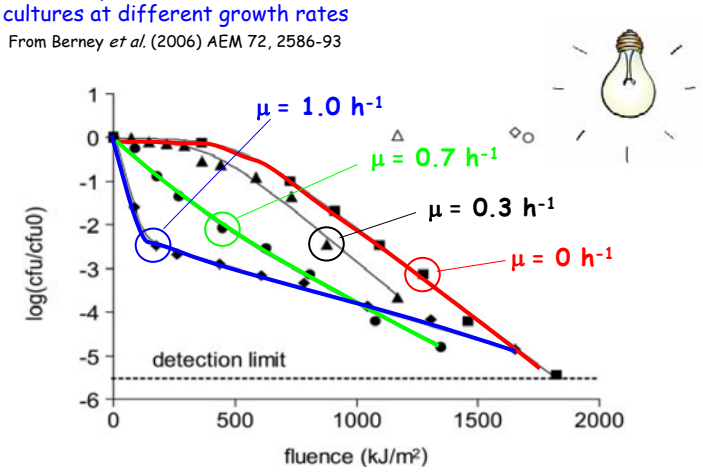
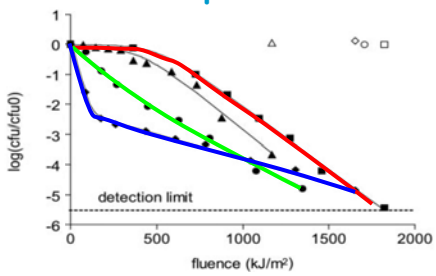
Sensitivity to UVA irradiation of *E. coli* harvested from LB chemostat cultures at different growth rates

From Berney *et al.* (2006) AEM 72, 2586-93



Inactivation curves modelled and statistically assessed with the Geeraerd/van Impe modelling tool (Geeraerd et al. 2005, Int J Food Microbiol 102, 95-105).

Extrapolation from F_{90} to F_{99} etc. is difficult due to differing inactivation patterns



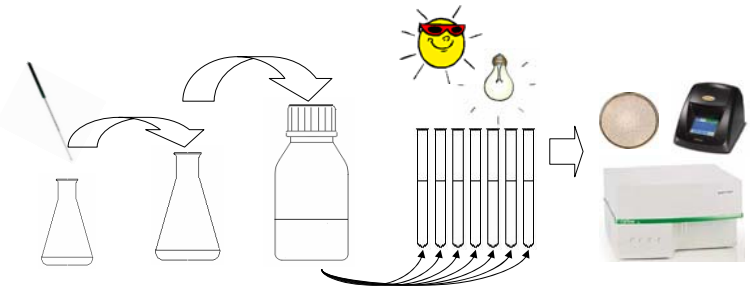
Strain	T_{90} (min)	F_{90} (kJ m^{-2})*	F_{99} (kJ m^{-2})*
E. coli MG1655	182 ± 15	1210 ± 188	1530 ± 70
S. Typhimurium	187 ± 37		
S. flexneri ATCC 12022	136 ± 37	932 ± 233	1194 ± 142
V. cholerae 01 Ogawa biotype E1 Tor	24 ± 5		

*Fluence was calculated from solar irradiation data for the wavelength range between 350 and 450 nm.

From Berney *et al.* (2006) J Appl Microbiol 101, 828-836

21

…therefore: Standard procedure for growt of cells used in SODIS experiments



Loop inoculation
⇩
Growth to exponential phase in LB medium OD_{546}= 0.1-0.2

Dilution in fresh LB medium
⇩
Growth to stationary phase (18 h after transfer)

Harvest by centrifug.
⇩
Dilution to 10^7 cells/ml in 0.22µm-filtered Evian

Irradiation

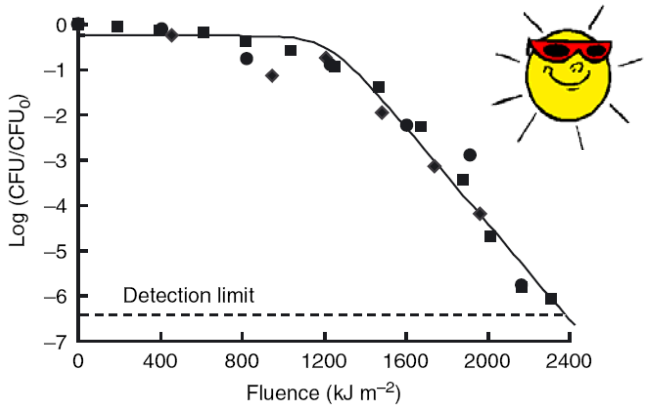
Determination of
- culturability
- ATP
- viability
- etc.

LB medium: 10g tryptone, 10g yeast extract, 10g NaCl, diluted to 33% with ultrapure water

22

Standard procedure results in good reproducibility of data for *E. coli*

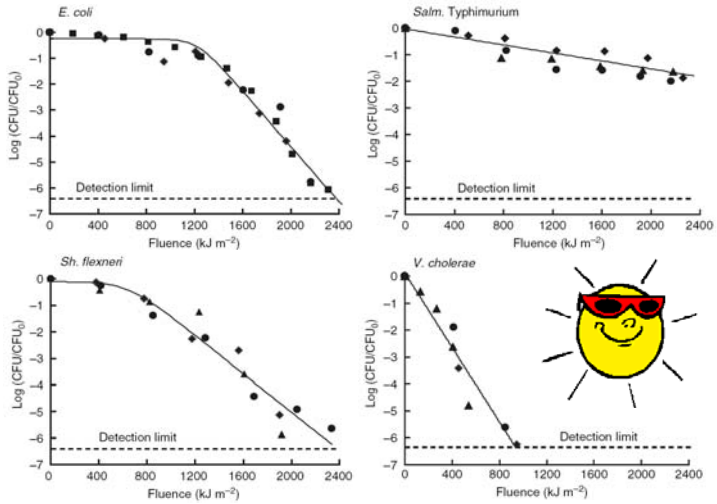
Inactivation curve of *E. coli* exposed to sunlight (350-450nm) on three different days (●, ◆, ■) at 37°C.



From Berney *et al.* (2006) J Appl Microbiol 101, 828-836

...and also for other pathogens

Inactivation curves of *E. coli, S. Typhimurium, S.flexneri and V. cholerae* exposed to sunlight (350-450nm) on three different days (●, ◆, ■) at 37°C



From Berney *et al.* (2006) J Appl Microbiol 101, 828-836

Applicability of reciprocity law within range of natural fluence rates (~50-1000 Wm^{-2})

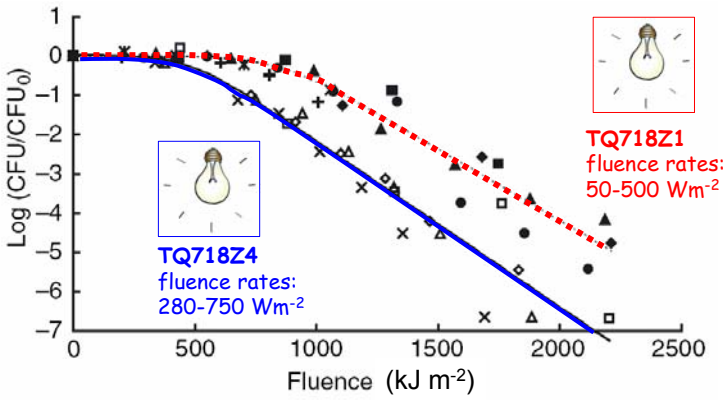


Figure 3 Inactivation curves of *Escherichia coli* exposed to 11 different UVA intensities [TQ150: (+) 56 and (■) 91 W m^{-2}; TQ718 Z1: (✕) 352, (●) 481 W m^{-2}, (◆) 542 W m^{-2}, (▲) 569 W m^{-2}; TQ718 Z4: (×) 282, (◇) 305, (△) 314 and (□) 734 W m^{-2}].

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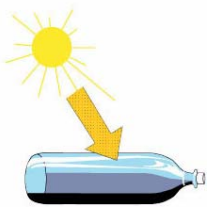
Today's focus



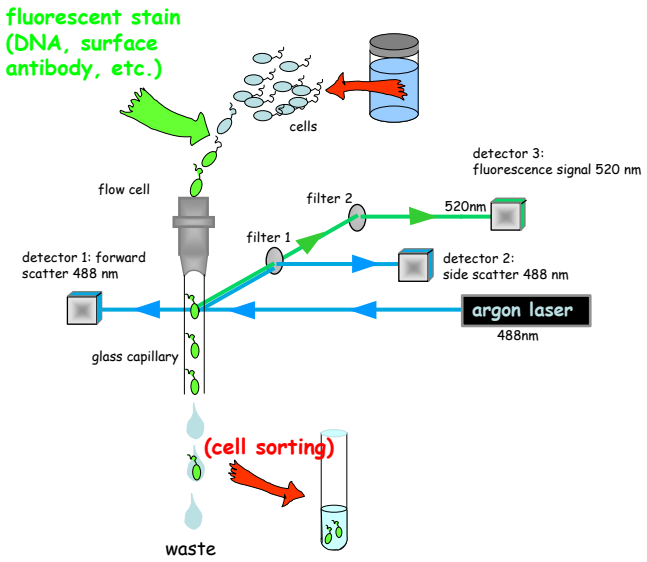
1) Importance of pre-cultivation

2) Importance of using several parameters simultaneously for assessment of cell viability

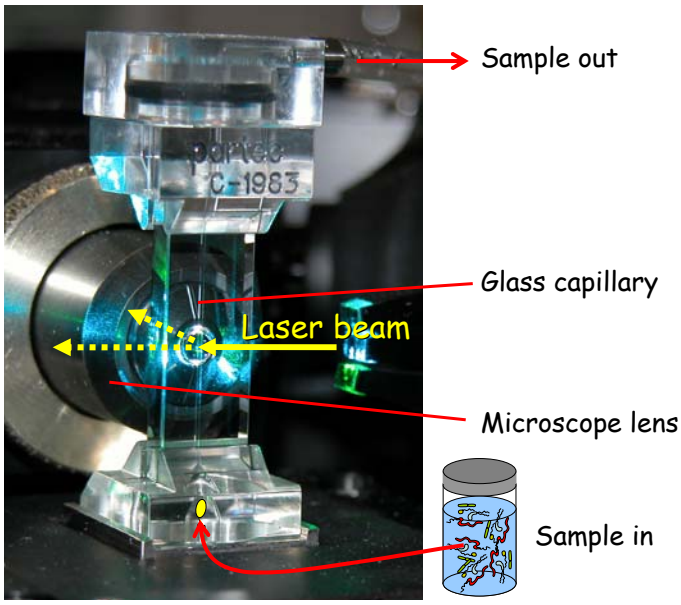
3) Use of flow cytometry-based methods in combination with conventional methods

4) Damage induced by (artificial) solar radiation

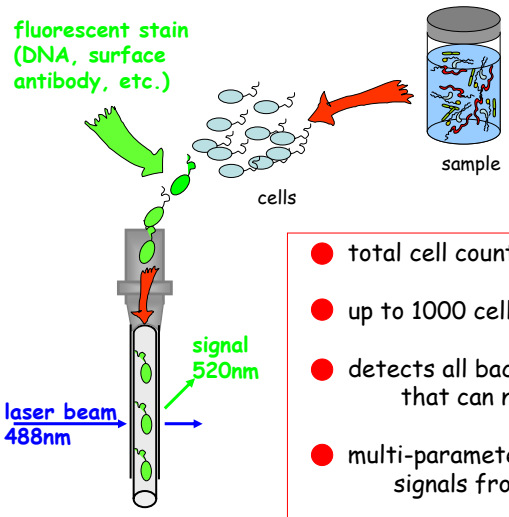
The principle of flow cytometry



Measuring cuvette (flow cell)



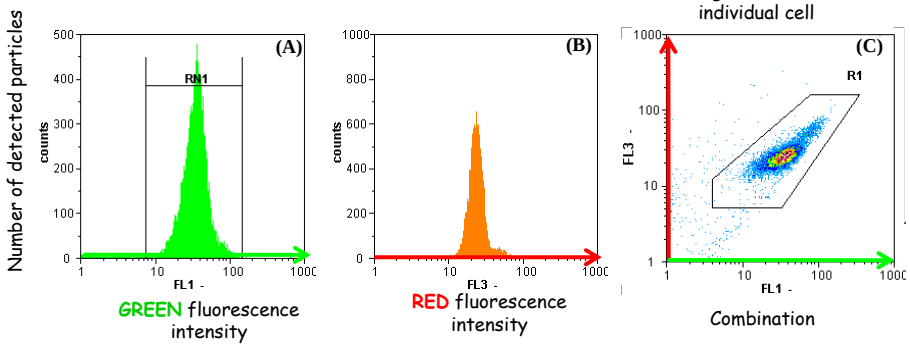
Advantages of flow cytometry



- total cell count in < 15 minutes
- up to 1000 cells/second counted
- detects all bacteria, also those that can not be cultured
- multi-parameter analysis (multiple signals from a single cell)
- automatable

Graphical presentation of the result

Each point is a signal from an individual cell



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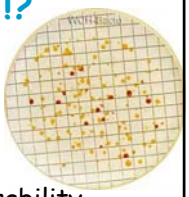
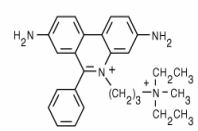
Take-home messages

31

Dead or alive?
A question of the method used !?

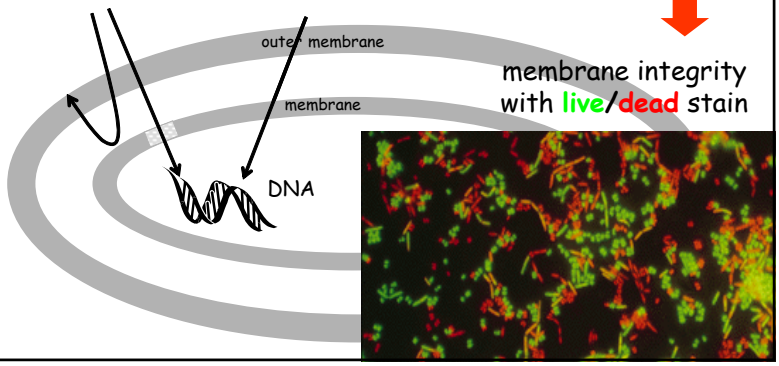


culturability



propidium iodide
red fluorescent

Syto®9
green fluorescent
(structure?)



membrane integrity
with live/dead stain

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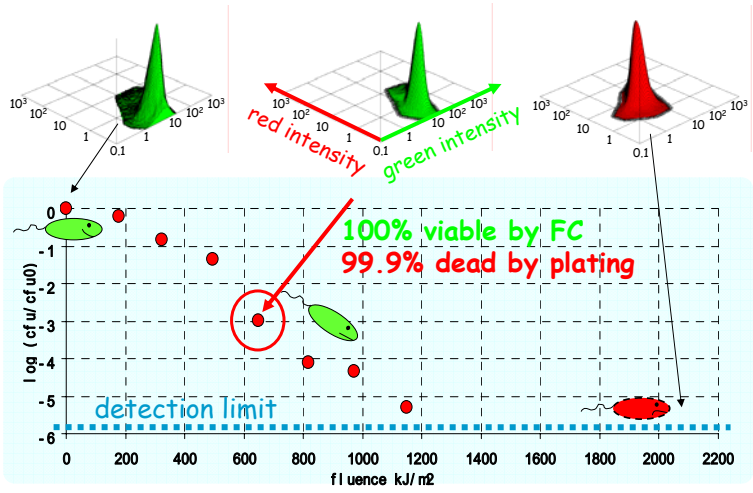
Take-home messages

32

Viability assessment during SODIS using plating and live/dead stain



Viability assessment of *E. coli* during SODIS using FCM (live/dead stain)

- Introduction and overview light exposure systems
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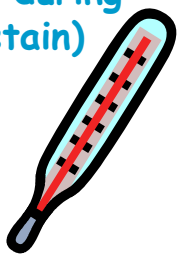
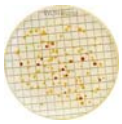
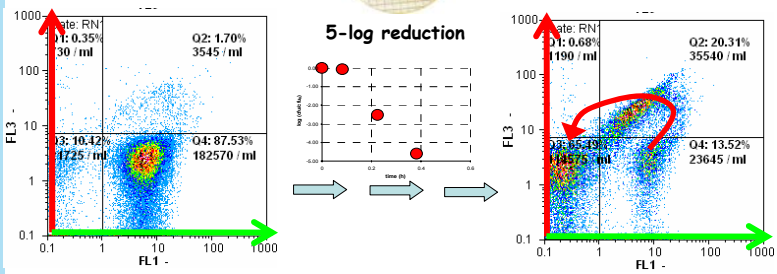
Temperature only (48°C)







Viability assessment of *E. coli* during SODIS using FCM (live/dead stain)

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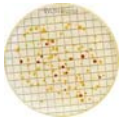
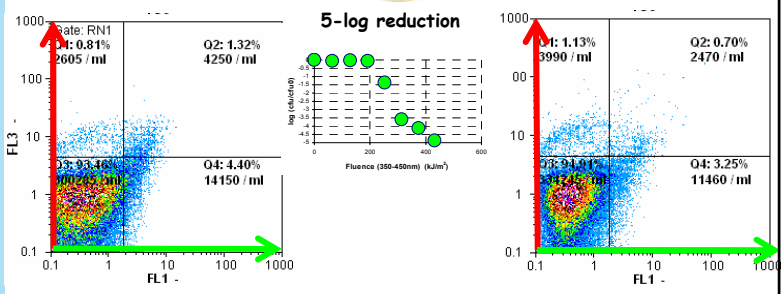
Sunlight only (20°C)





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Used "tool kit" for assessing cell viability and death during exposure to sunlight

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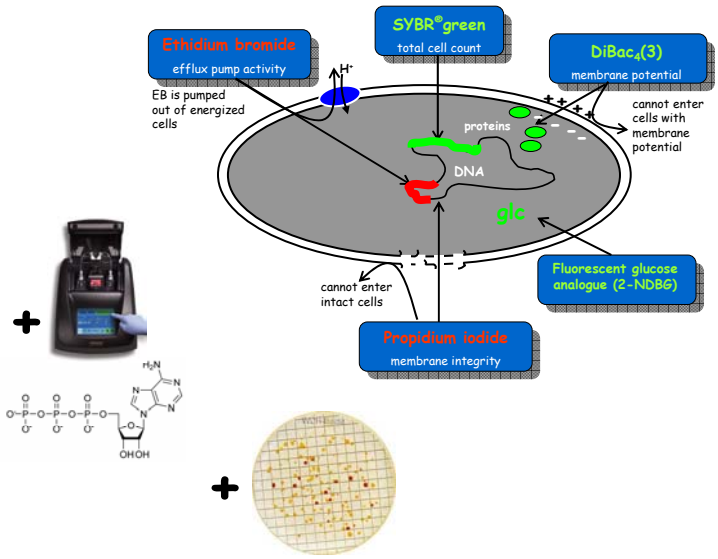
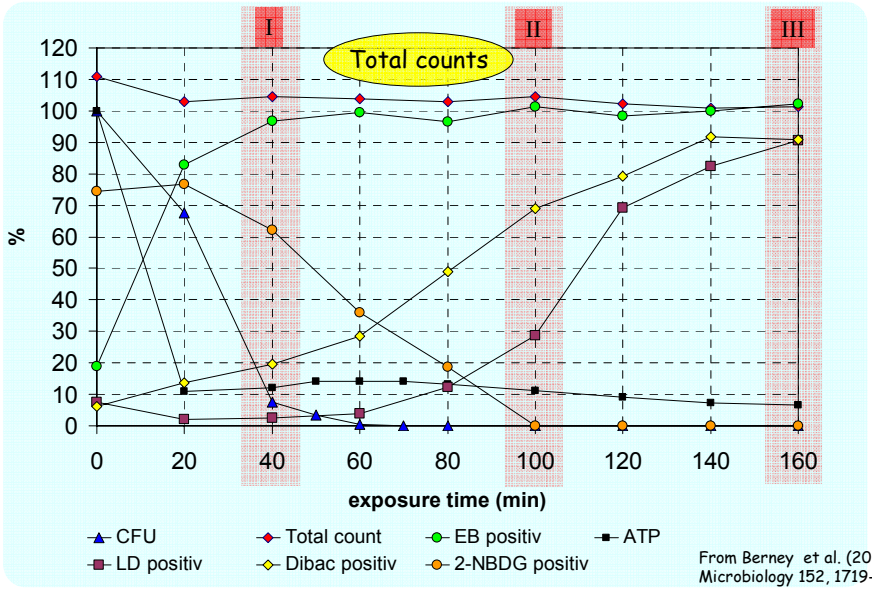
35

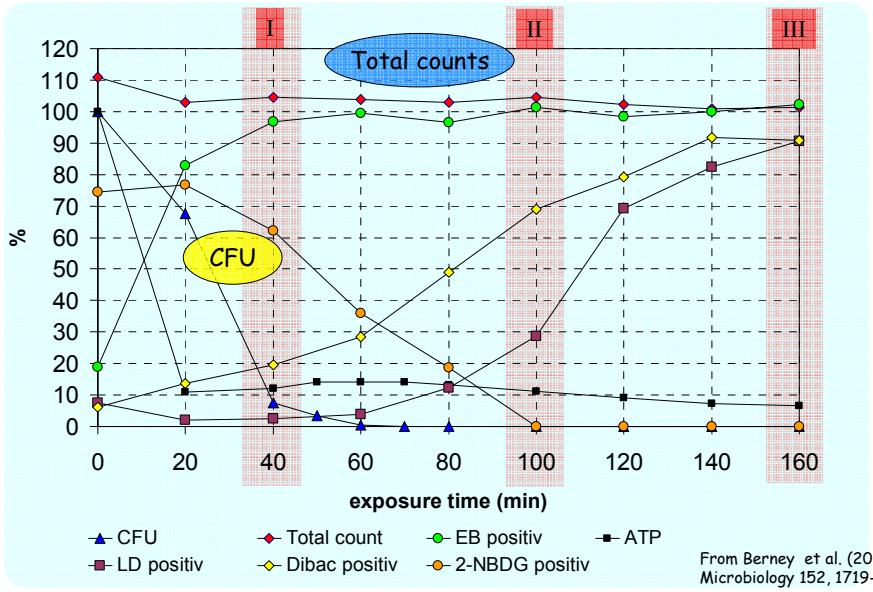


The "agony" of *E. coli* during solar stress



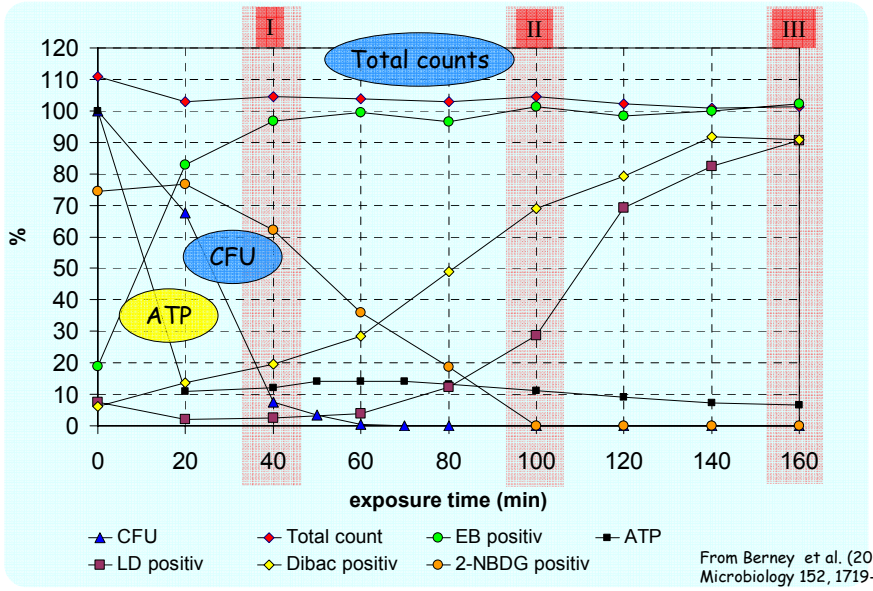
From Berney et al. (2006) Microbiology 152, 1719-29

The "agony" of *E. coli* during solar stress



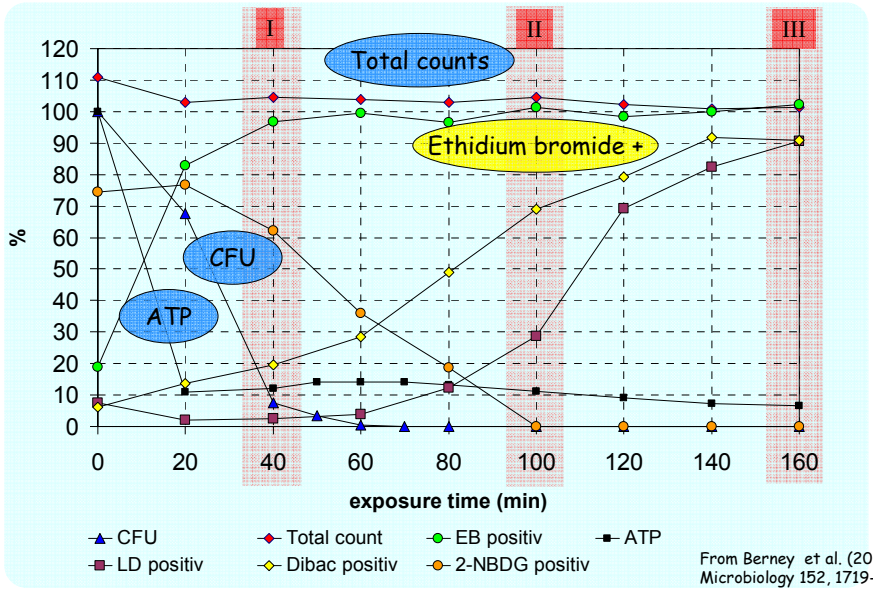
From Berney et al. (2006) Microbiology 152, 1719-29

The "agony" of *E. coli* during solar stress



From Berney et al. (2006) Microbiology 152, 1719-29

The "agony" of *E. coli* during solar stress



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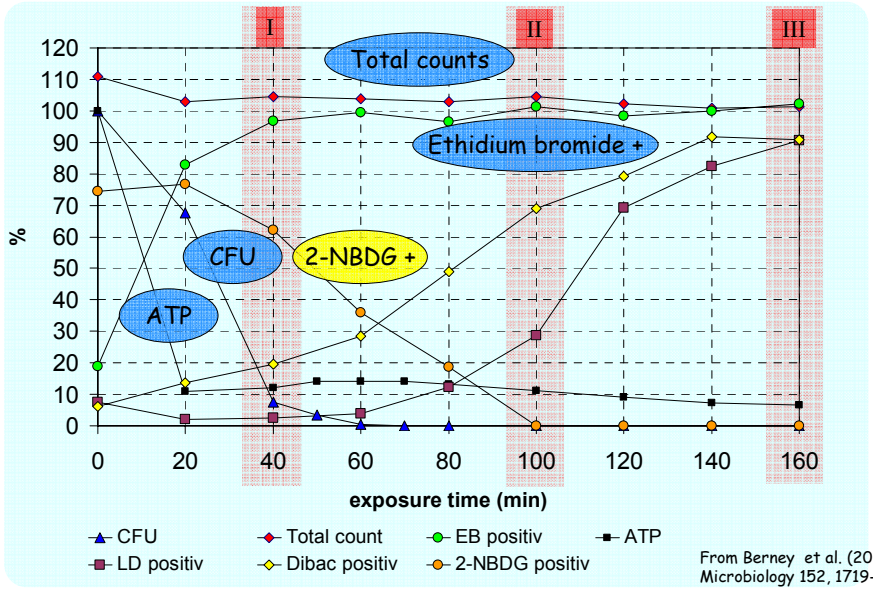
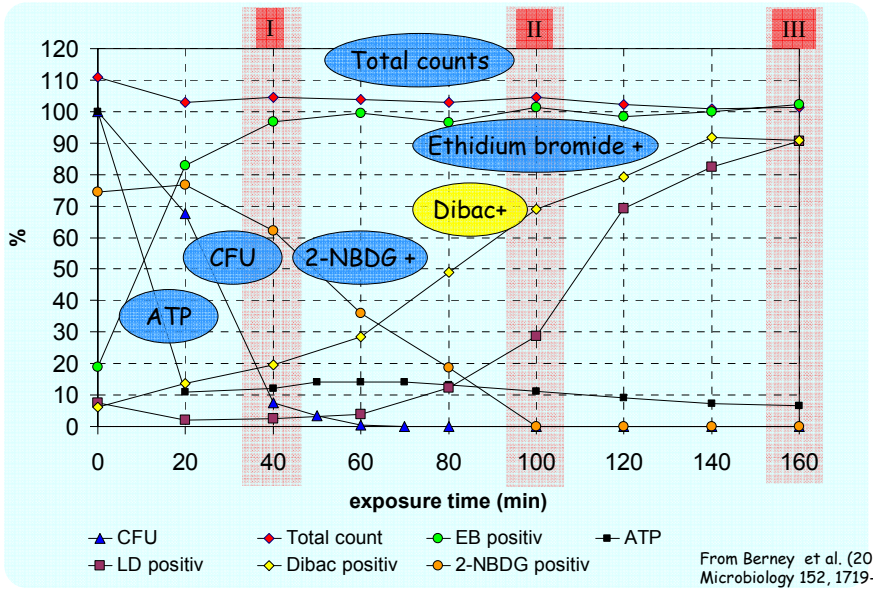
The "agony" of *E. coli* during solar stress



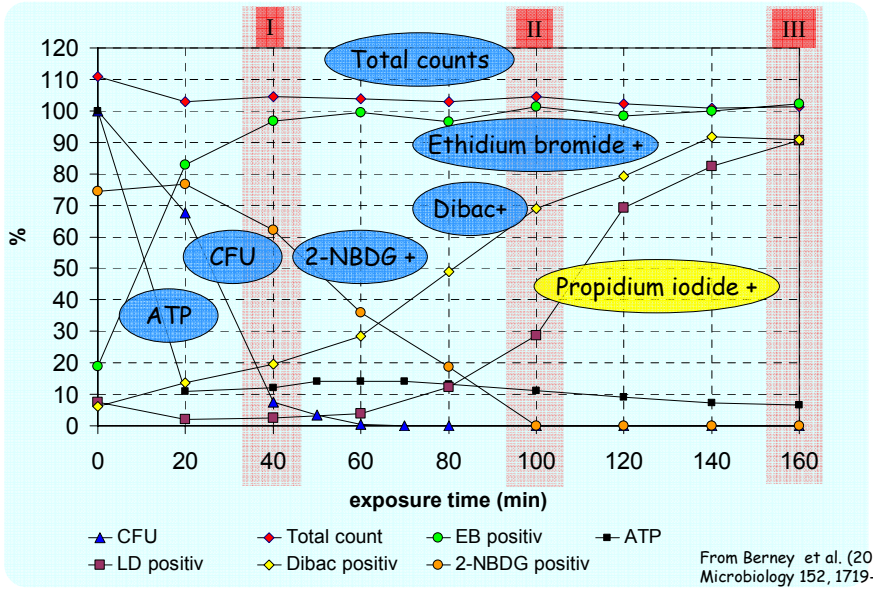
From Berney et al. (2006) Microbiology 152, 1719-29

The "agony" of *E. coli* during solar stress



From Berney et al. (2006) Microbiology 152, 1719-29

The "agony" of *E. coli* during solar stress

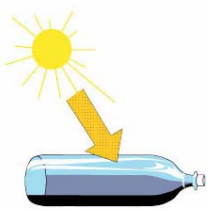


From Berney et al. (2006) Microbiology 152, 1719-29

The "agnoy" of *E. coli* during SODIS: Multiparameter assessment of viability

	Start	State I	State II	State III
Culturability	+	-	-	-
Total ATP	high	low	low	low
Efflux pump activity	+	-	-	-
Repair / recovery	+	-	-	-
Membrane potential	+	+	-	-
Glucose transport	+	+	-	-
Membrane integrity	+	+	+	-

Today's focus



1) Importance of pre-cultivation

2) Importance of using several parameters simultaneously for assessment of cell viability

3) Use of flow cytometry-based methods in combination with conventional methods

4) Damage induced by (artificial) solar radiation

45

Experiments suggest:

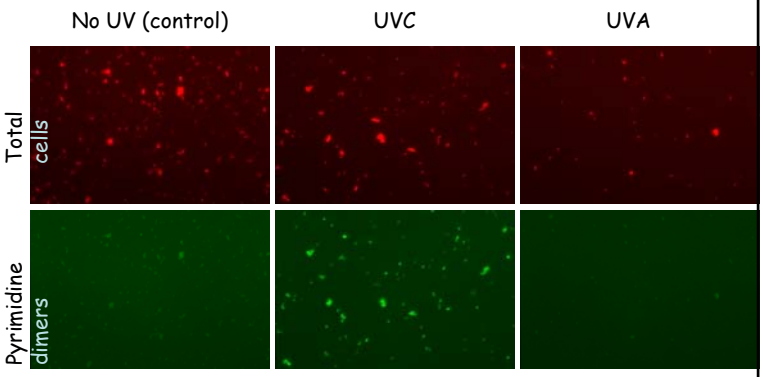
"something" happens first at the membrane and energy metabolism

Is protein damage responsible (not the DNA)?

46

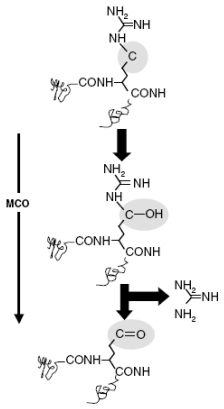
Little DNA damage from UVA

T-T dimers: UV-C induced T-T cyclobutane rings are detected with specific fluorescent antibodies in fluorescence microscope. Should be also detectable in flow cytometer.



(pictures from C. Bassin)

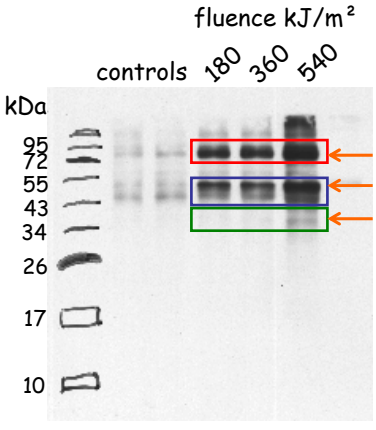
"Aging" of proteins: carbonylation



1. Irradiation causes **Metal Catalyzed Oxidation (MCO)** on proline, arginine, lysine and threonine.
2. Cell lysis
3. Derivatization on carbonylated sites
4. Electrophoresis and blotting of proteins
5. Immunodetection of the hydrazone bound to carbonylated sites

Bosshard et al. FEMS Microbiology Meeting, Göteborg, June 2009

Protein carbonylation during SODIS



- Increase of carbonylation is detected at very **low fluences**.

-> rapid carbonylation of 2 protein bands

-> appearance of a third band at 540 kJ/m²

-> 3 MW ranges of interest:

72- 95 kDa
43- 55 kDa
34- 43 kDa

- Pattern is **reproducible**

-> Carbonylation targets specific proteins

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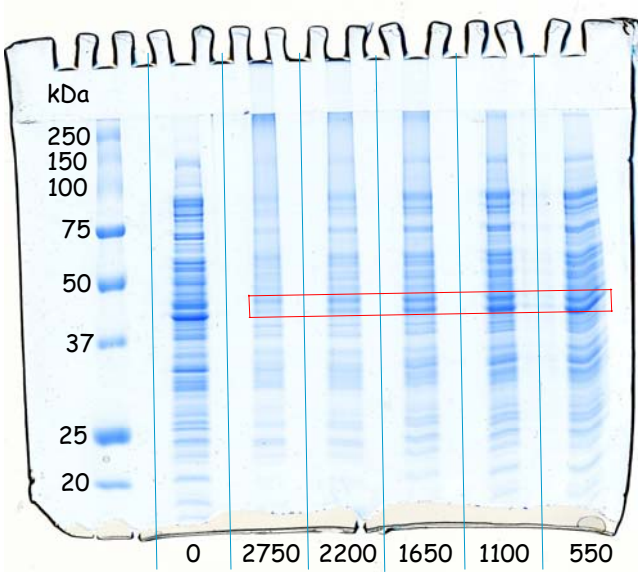
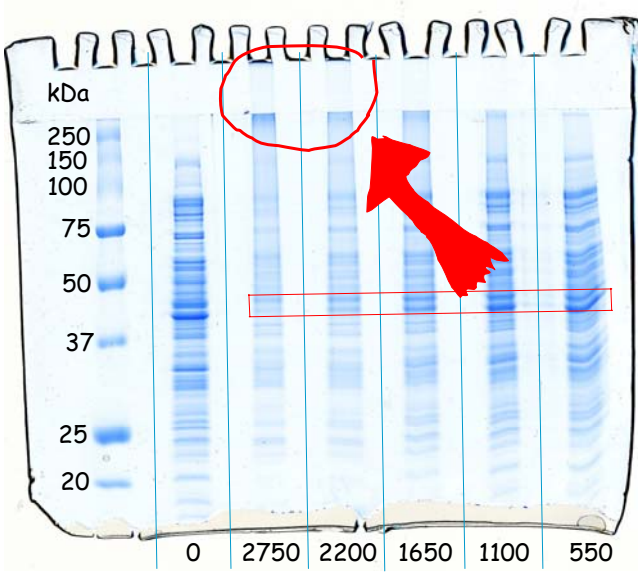
Loss of protein due to SODIS?



49

Loss of protein due to SODIS?



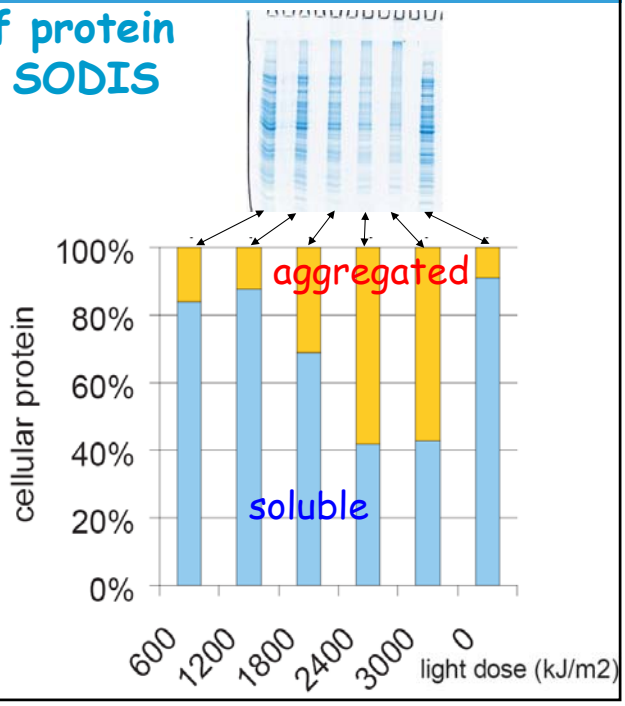
50

eawag aquatic research

Loss of protein due to SODIS

- Introduction and overview light exposure systems
- Importance of cellular state
- Multi-parameter assessment of microbial viability and death
- Membrane proteins as primary targets
- Take-home messages

51



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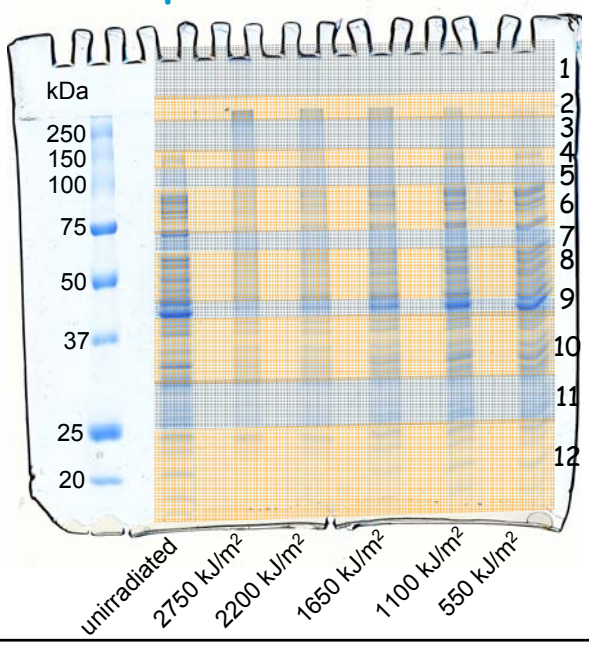
MSMS-assisted protein identification

1. Irradiation of cells
2. Cell lysis
3. Electrophoresis, cutting gel into slices
4. In-gel-digestion of proteins to peptides for MSMS
5. Identifying of proteins by comparison with data base
6. „Quantifying" proteins by counting their peptides



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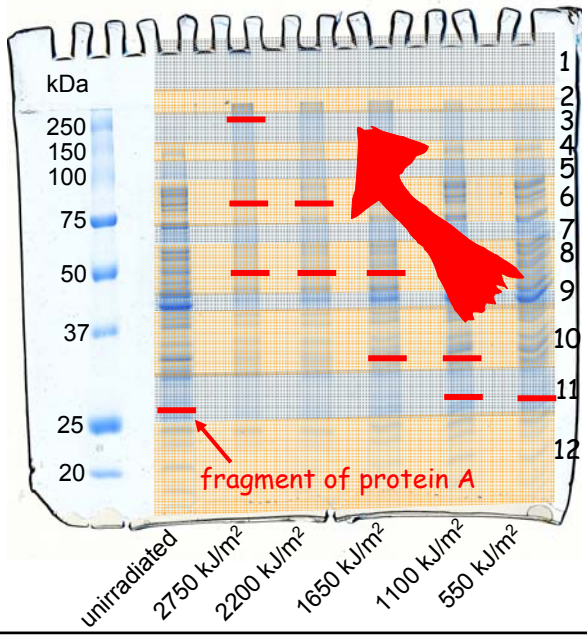
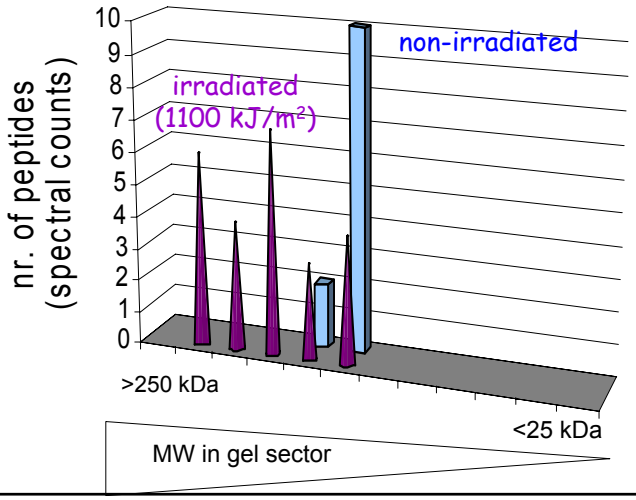
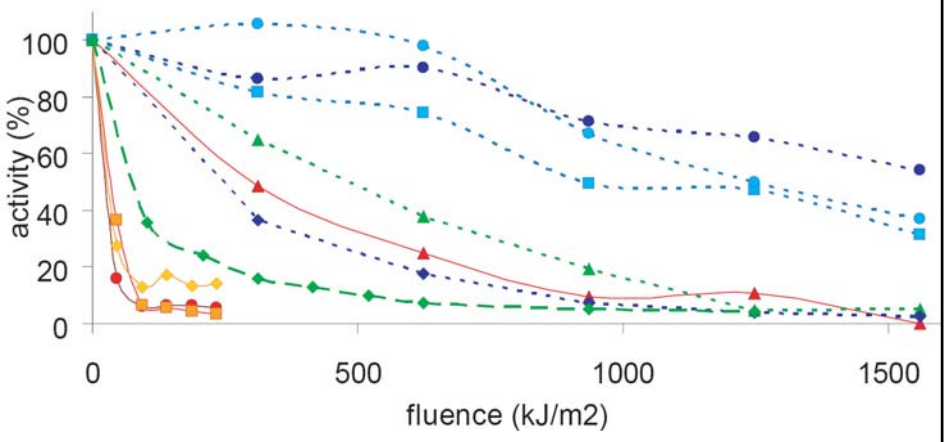


Example of fragment moving in gel

A membrane protein of the respiratory chain:
NADH-quinone oxidoreductase subunit G; size 100 kDa



Activities of selected enzymes



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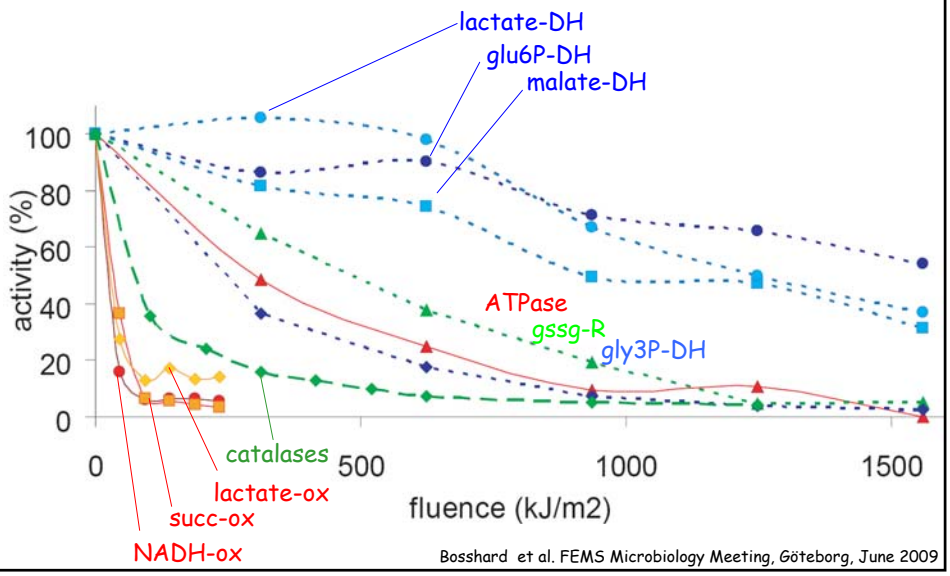
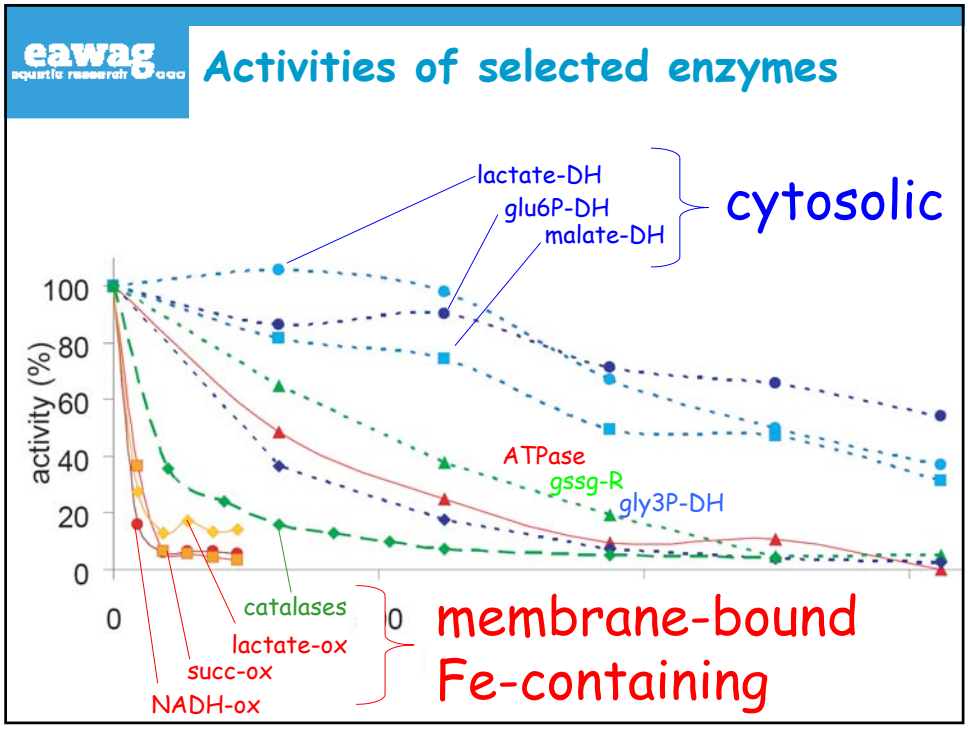
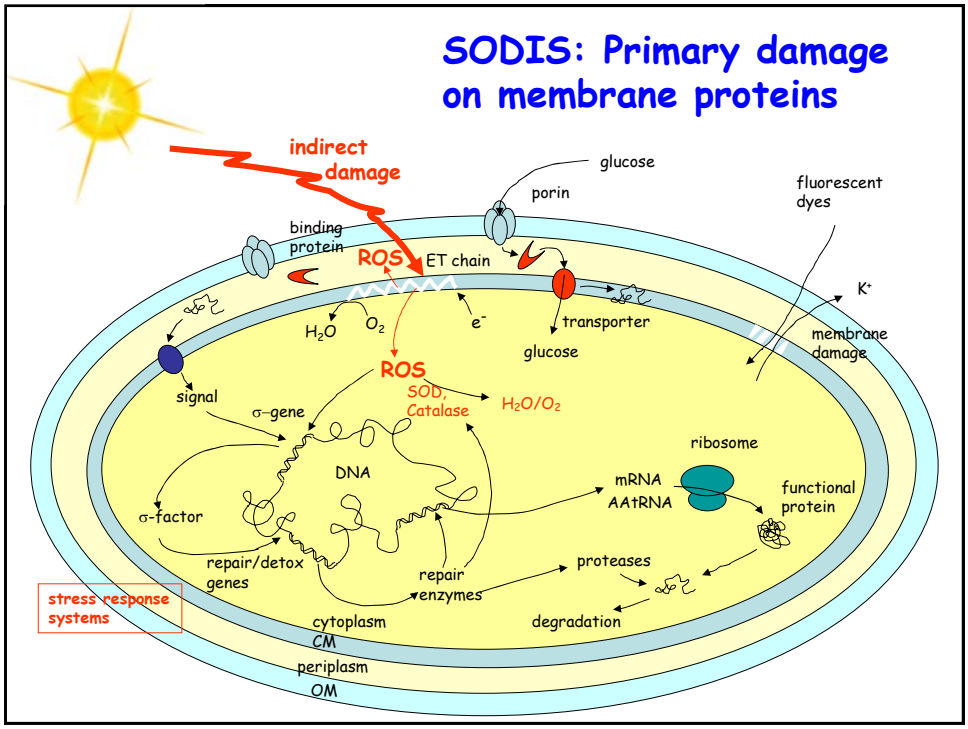
Activities of selected enzymes



Bosshard et al. FEMS Microbiology Meeting, Göteborg, June 2009


eawag
aquatic research
Activities of selected enzymes
lactate-DH
glu6P-DH
malate-DH
cytosolic
activity (%)
100
80
60
40
20
0
0
0
ATPase
gssg-R
gly3P-DH
catalases
lactate-ox
succ-ox
NADH-ox
membrane-bound
Fe-containing


SODIS: Primary damage on membrane proteins
indirect damage
glucose
porin
fluorescent dyes
binding protein
ROS
ET chain
K+
H2O
O2
e-
transporter
glucose
membrane damage
ROS
SOD, Catalase
H2O/O2
signal
σ-gene
ribosome
DNA
mRNA
AAtRNA
functional protein
σ-factor
repair/detox genes
repair enzymes
proteases
degradation
stress response systems
cytoplasm
CM
periplasm
OM

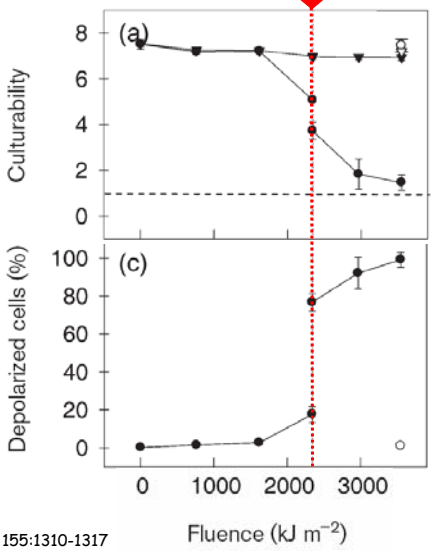
Damage continues...

- Introduction and overview light exposure systems
- Importance of cellular state
- Multi-parameter assessment of microbial viability and death
- Membrane proteins as primary targets
- Take-home messages

59

Salmonella typhimurium

night break 16h - 09h



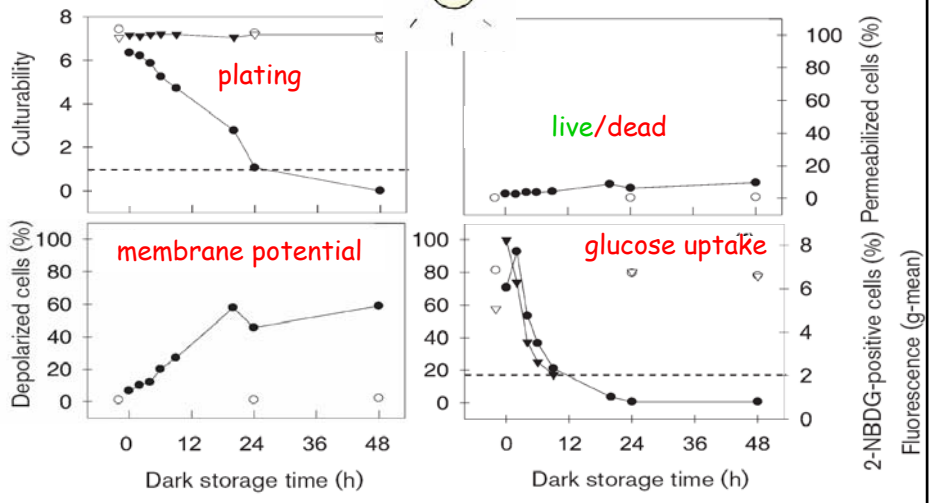
Bosshard et al. (2009) Microbiology, 155:1310-1317

Damage continues during dark storage

Salmonella typhimurium

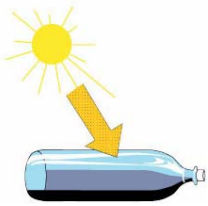
1500 kJ/m² (205 W/m² for 2h)

dark storage



Bosshard et al. (2009) Microbiology, 155:1310-1317

Take-home messages



1) SODIS works

2) Sensitivity of microbes is dependent on growth conditions (-> reproducibility!)

3) Combination of methods should be used to assess microbial viability/death

4) Primary target of sunlight is protein, inner membrane proteins are damaged first, most likely by ROS generated at the respiration chain

5) Damage continues after exposure, seem not to be able to repair damage

Thanks!

Michael Berney
Hansueli Weilenmann
Franziska Bosshard
Martin Wegelin
Regula Meierhofer
Frederik Hammes
Silvio Canonica

eawag aquatic research

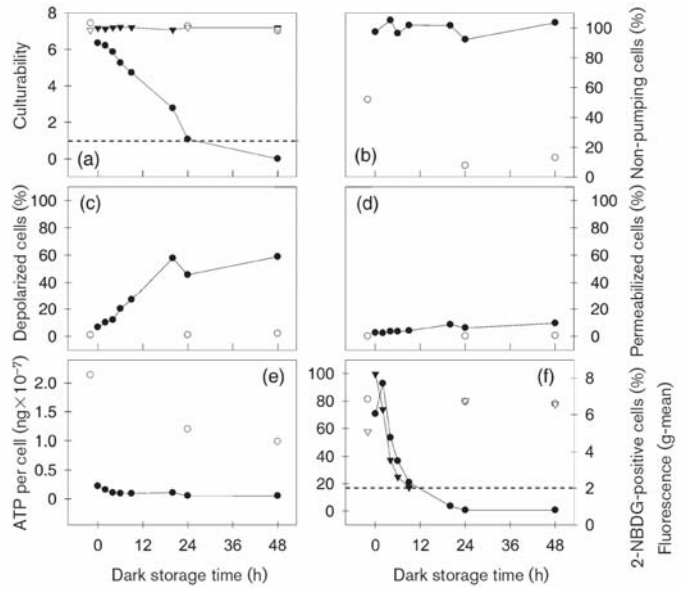
63

64

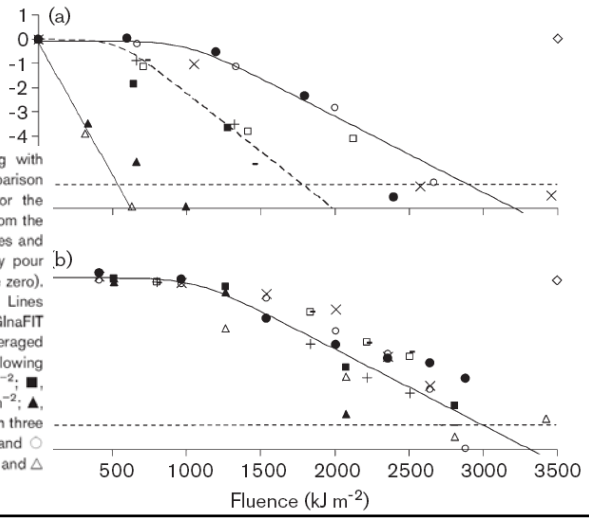
65

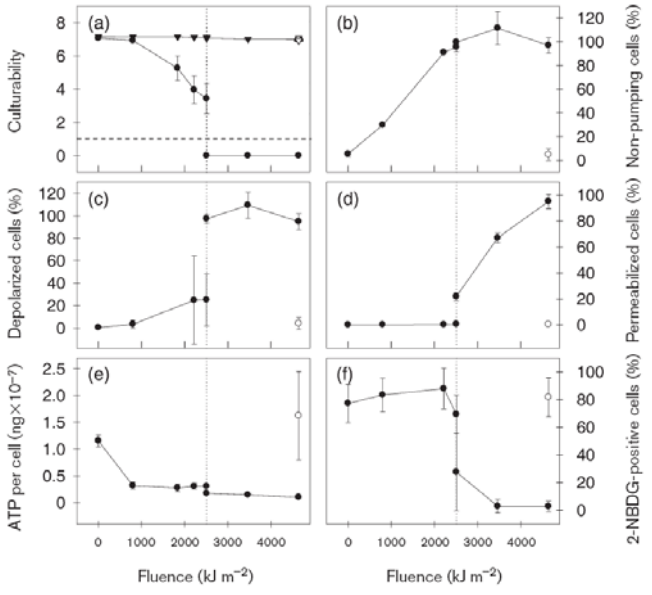
66

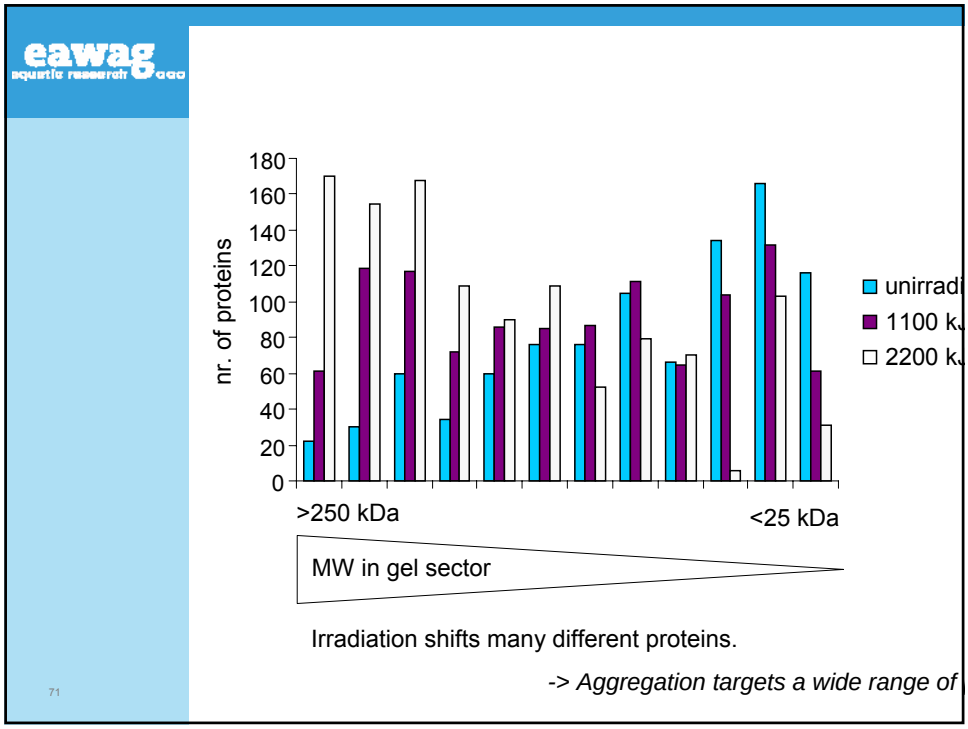
Appendix

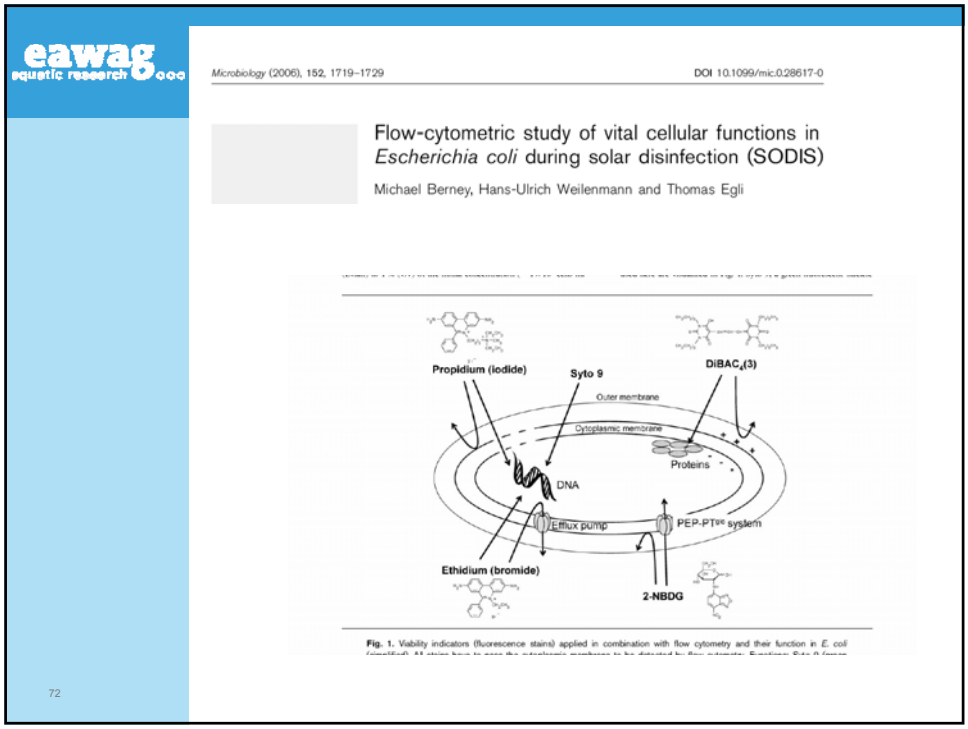

Culturability
(a)
(b)
Depolarized cells (%)
(c)
(d)
Permeabilized cells (%)
Non-pumping cells (%)
ATP per cell (ng×10⁻⁷)
(e)
(f)
2-NBDG-positive cells (%)
Fluorescence (g-mean)
0 12 24 36 48
Dark storage time (h)
0 12 24 36 48
Dark storage time (h)

Deviations from light dose reciprocity appearing with
t intensities of artificial UVA light irradiation in comparison
, low-intensity irradiation with sunlight, shown for the
bility of *Sh. flexneri*. Bacterial cells were harvested from the
ry phase of an LB batch culture, washed three times and
in bottled mineral water. C.f.u. were measured by pour
and sensitivity was recorded as c.f.u./(c.f.u. at time zero).
tal dashed lines indicate the detection limits. Lines
nt modelled inactivation curves with the program GInaFIT
erd *et al.*, 2005). Empty diamonds (◇) represent averaged
s. (a) Artificial UVA light was applied at the following
es: ×, 163 W m^{-2}; ●, 332 W m^{-2}; ○, 370 W m^{-2}; ■,
m^{-2}; +, 732 W m^{-2}; □, 786 W m^{-2}; -, 802 W m^{-2}; ▲,
m^{-2} and △, 1315 W m^{-2}. (b) Sunlight irradiation on three
t days in biologically independent triplicates: ×, ● and ○
ugust 2006; ■, + and □ on 31 August 2006; -, ▲ and △
uly 2006.

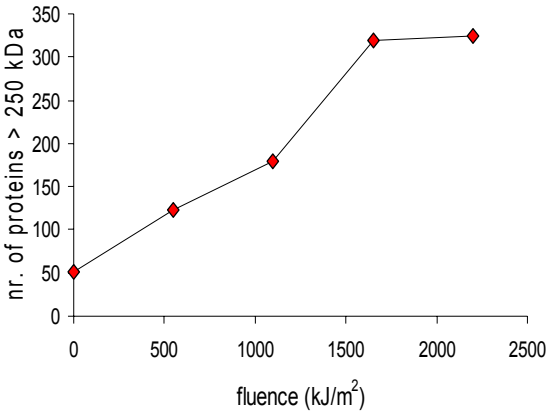





eawag
aquatic research
180
160
140
120
100
80
60
40
20
0
nr. of proteins
unirradi
1100 k
2200 k
>250 kDa
<25 kDa
MW in gel sector
Irradiation shifts many different proteins.
-> Aggregation targets a wide range of
71


eawag
aquatic research
Microbiology (2006), 152, 1719–1729
DOI 10.1099/mic.0.28617-0
Flow-cytometric study of vital cellular functions in Escherichia coli during solar disinfection (SODIS)
Michael Berney, Hans-Ulrich Weilenmann and Thomas Egli
Propidium (Iodide)
Syto 9
DiBAC4(3)
Outer membrane
Cytoplasmic membrane
Proteins
DNA
Efflux pump
PEP-PTsys system
Ethidium (bromide)
2-NBDG
Fig. 1. Viability indicators (fluorescence stains) applied in combination with flow cytometry and their function in E. coli
72

/ (>250



The number of identified proteins increases **7-fold** during irradiation!

…where do they come from?

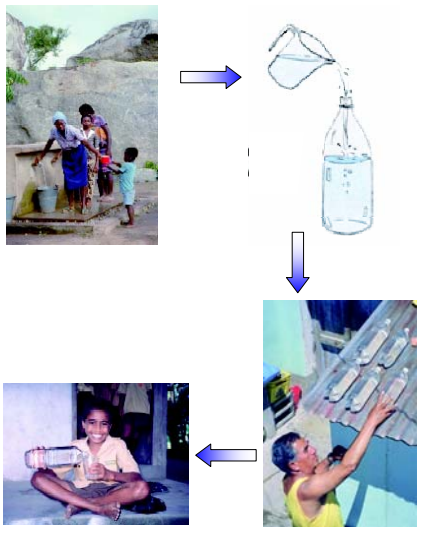
73

effective

SODIS – application
- needs little infrastructure
- prevents diarrhea

SODIS – effect
- improves microbiological water quality
- by solar UVA light and temperature

SODIS – limitations
- turbid waters
- bad chemical water quality



74

Field experiments with PET bottles

Introduction and overview light exposure systems

Importance of cellular state

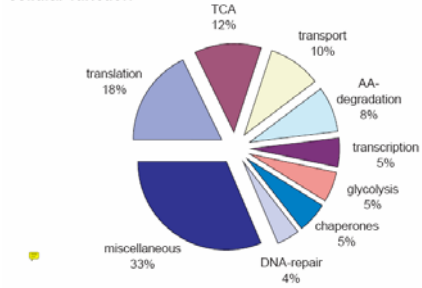
Importance of multi-parameter assessment and use of flow cytometry

Summary & outlook

75

Title

When analysing gels by MSMS, we found some proteins with a higher molecular weight than expected. These shifts based on aggregation damage to the proteins were reproducible. Proteins with this behaviour are depicted for their cellular function



76

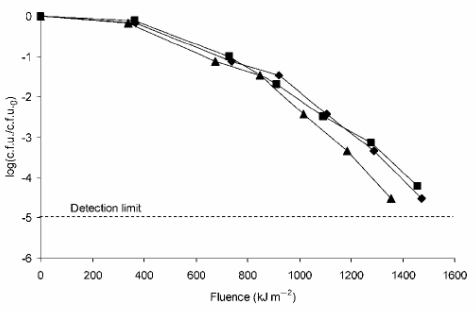


Fig. 2. Inactivation curves of *E. coli* K-12 MG1655 exposed to artificial UVA light in three independent experiments (▲, ■, ◆). Bacterial cells were harvested from a stationary-phase LB batch culture, washed and diluted in mineral water (Evian). Initial cell numbers in these experiments were $1{\cdot}7–1{\cdot}9 \times 10^7$ cells ml^{-1} Culturability of bacterial cells was measured with the pour-plate method using TSA.

77

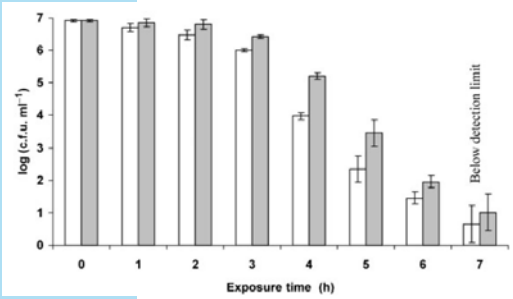


Fig. 3. Culturability of *E. coli* K-12 MG1655 during sunlight exposure for 7 h. Bacterial cells were harvested from a stationary-phase LB batch culture, washed and diluted in mineral water (Evian). Samples were plated either on unsupplemented TSA (pour-plate method) and incubated under aerobic conditions (open bars), or on TSA with 0·05 % sodium pyruvate and incubated in anaerobic jars (grey bars). Error bars represent SD from triplicate measurements.

78

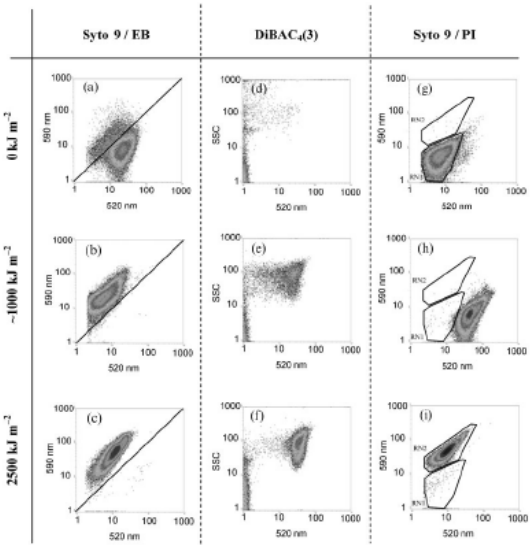


Fig. 5. Flow-cytometric analysis of *E. coli* K-12 MG1655 irradiated with artificial UVA light. Bacterial cells were harvested from a stationary-phase LB batch culture, washed and diluted in mineral water (Evian). Bacterial cell samples were stained with Syto 9/EB, $DiBAC_4(3)$ or Syto 9/PI and analysed on a flow cytometer after being exposed to different fluences (irradiation intensity × time). After 1000 kJ m^{-2}, > 95% of the cells were non-pumping (b), 40% were depolarized (e), and < 1% permeabilized (upper polygon-gate RN2) (h). After 2500 kJ m^{-2}, 100% of the cells were non-pumping (c), 100% depolarized (f), and > 90% permeabilized (polygon-gate RN2) (i). SSC, side scatter.

79

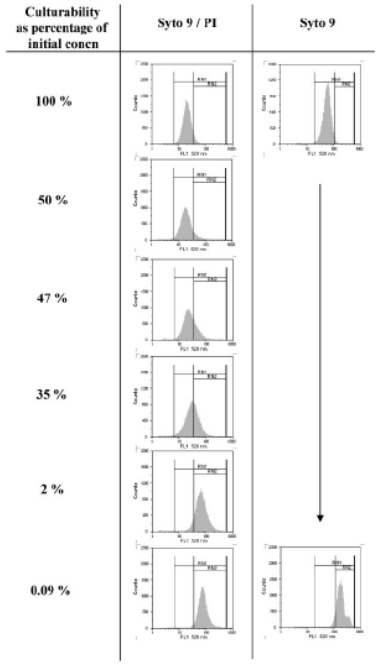
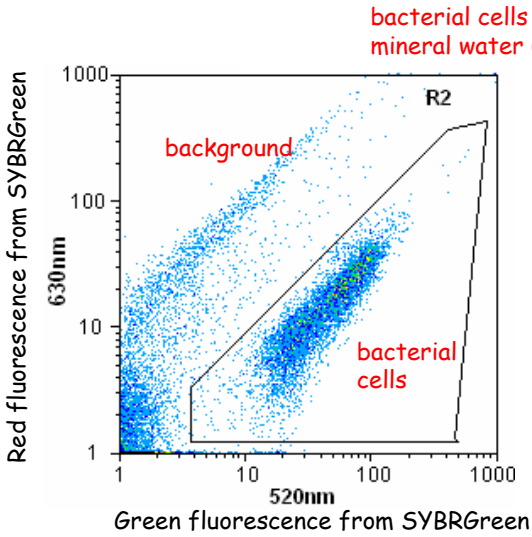


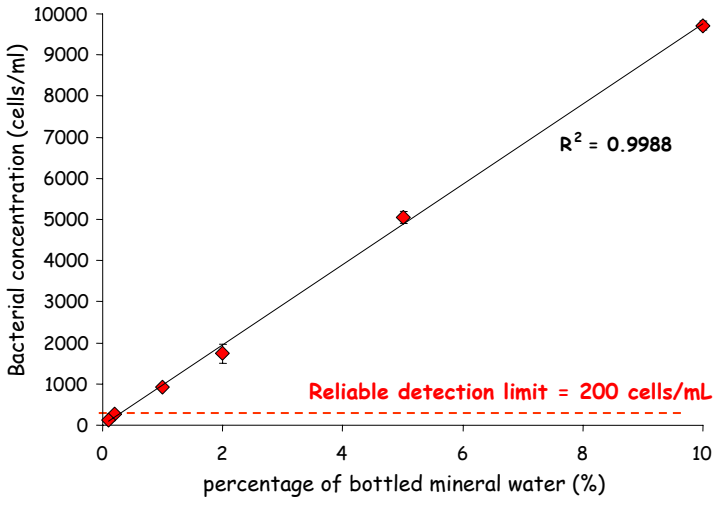
Fig. 7. Flow-cytometric analysis of *E. coli* K-12 MG1655 irradiated with artificial UVA light. Bacterial cells were harvested from a stationary-phase LB batch culture, washed and diluted in mineral water (Evian). Staining with Syto 9/PI or Syto 9 alone was compared to culturability on TSA (pour-plate method). The loss of culturability coincided with an increase in green fluorescence intensity (range-gate RN2).

80

An easy task: Counting bacterial cells (SYBRGreen-stained)

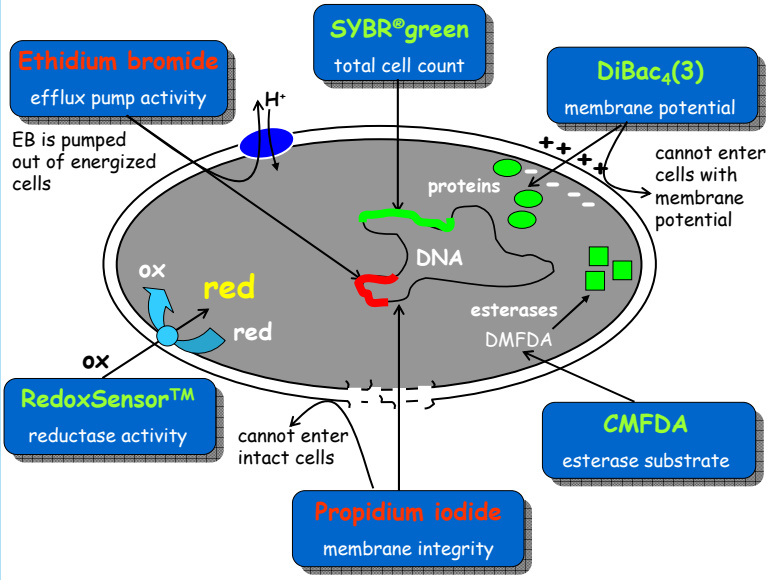


Flow cytometry detection limit: how low can you go?

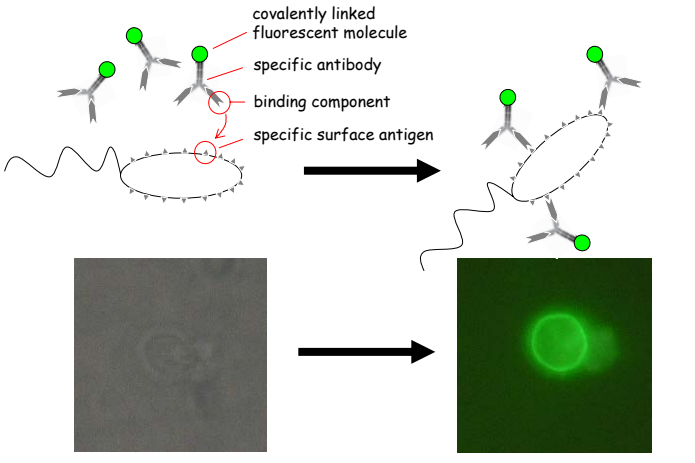


Hammes et al. (submitted)

Staining options: cellular constituents and physiological properties



Staining options: cellular constituents with fluorescent antibodies or probes



conventional microscopy of *Cryptosporidium* oocyst

fluorescence microscopy of *Cryptosporidium* oocyst

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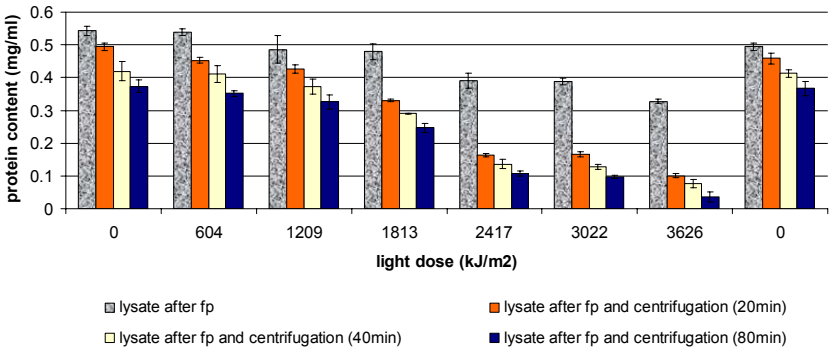
Protein a



85

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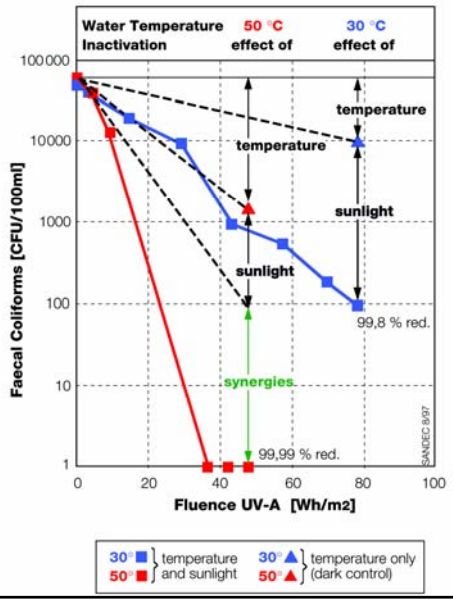
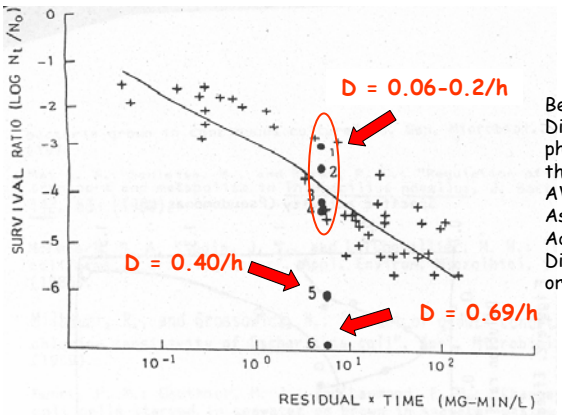
Inactivation synergies of heat and light

- Introduction and overview light exposure systems
- Importance of cellular state
- Importance of multi-parameter assessment and use of flow cytometry
- Summary & outlook



86

Increased stress resistance during slow growth for ClO_2



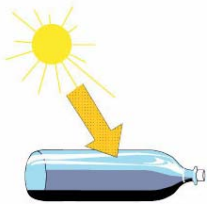
Berg JD., 1987. Disinfection: The physiological state of the test organism. In: AWWA Seminar on Assurance of Adequate Disinfection, or C-T or not C-T, pp.85-99.

Figure 4 Inactivation of naturally occuring fecal coliform bacteria by chlorine dioxide in filtered secondary-treated wastewater from Palo Alto, CA (A) and San Jose, CA (B), represented by (+) and yielding the regression lines. Chemostat-grown E. coli data are identified numerically with the following growth conditions: 1: D = 0.06 h^{-1}, 15°C; 2) D = 0.20 h^{-1}, 15°C; 3) D = 0.06 h^{-1}, 25°C; 4) D = 0.20 h^{-1}, 25°C; 5) D = 0.40 h^{-1}, 25°C; 6) 0.69 h^{-1}, 25°C. S_R = 0.16% nutrient broth in all cases. Fecal coliform data (+) from Roberts et al. (1980).

87

Overview of work done & in progress (1)

Effect of growth conditions before exposure on disinfection efficiency with different bacterial pathogens (*E. coli, Salmonella typhimurium, Shigella flexneri, Vibrio cholerae*)



Applied and Environmental Microbiology (2006) 72, 2586-2593

Specific Growth Rate Determines the Sensitivity of *Escherichia coli* to Thermal, UVA, and Solar Disinfection

Michael Berney, Hans-Ulrich Weilenmann, Julian Ihssen, Claudio Bassin, and Thomas Egli*

Journal of Applied Microbiology **101** (2006) 828–836

Efficacy of solar disinfection of *Escherichia coli*, *Shigella flexneri*, *Salmonella* Typhimurium and *Vibrio cholerae*

M. Berney, H.-U. Weilenmann, A. Simonetti and T. Egli

88

Overview of work done & in progress (2)

Assessing disinfection efficiency with different methods and better insight into "cell injury" and "cell death"

Microbiology (2006), 152, 1719–1729

Flow-cytometric study of vital cellular functions in *Escherichia coli* during solar disinfection (SODIS)

Michael Berney, Hans-Ulrich Weilenmann and Thomas Egli

APPLIED AND ENVIRONMENTAL MICROBIOLOGY (2007) 73, 3283-3290

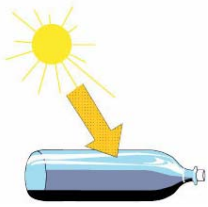
Assessment and Interpretation of Bacterial Viability by Using the LIVE/DEAD BacLight Kit in Combination with Flow Cytometry▽

Michael Berney,[1] Frederik Hammes,[1] Franziska Bosshard,[1,2] Hans-Ulrich Weilenmann,[1] and Thomas Egli[1,2]*

89

Overview of work done & in progress (3)

Investigation of cellular processes and ability to repair and adapt to light-induced cell damage



Environmental Microbiology (2006) 8(9), 1635–1647

Gene expression of *Escherichia coli* in continuous culture during adaptation to artificial sunlight

Michael Berney, Hans-Ulrich Weilenmann and Thomas Egli*

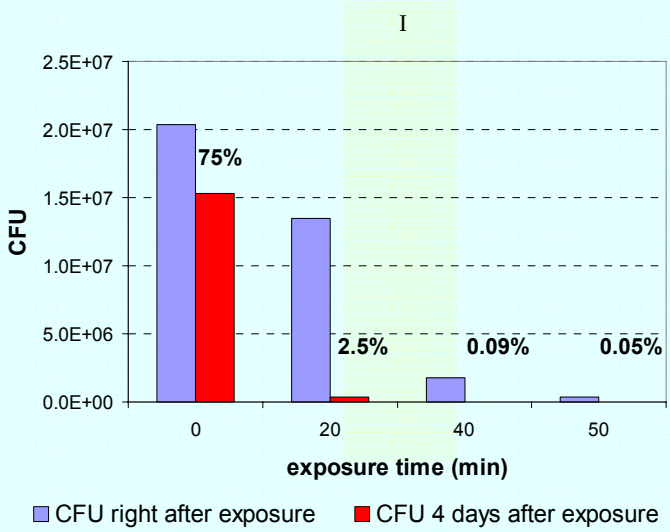
Journal of Photochemistry and Photobiology B: Biology 86 (2007) 149–159

Adaptation to UVA radiation of *E. coli* growing in continuous culture

Michael Berney, Hans-Ulrich Weilenmann, Thomas Egli *

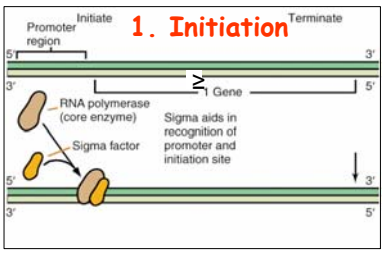
90

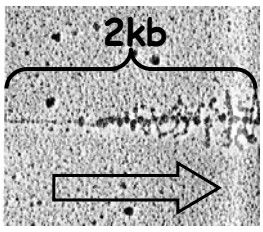
Recovery / repair after 4 days

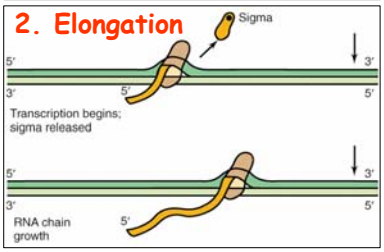


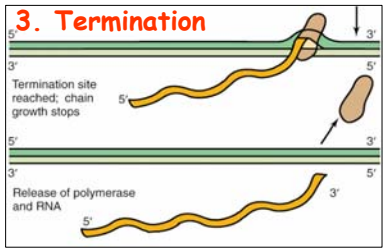
91

Role of sigma-factors in transcription in bacteria: recognition of specific promoter regions









92

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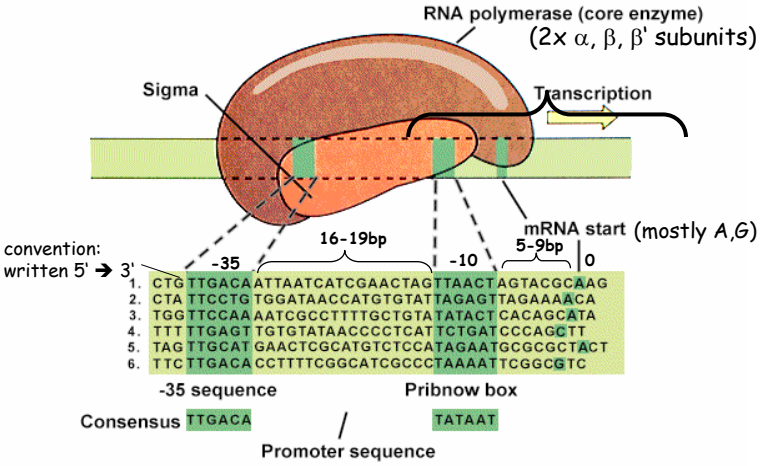

Promoter
- site where RNA polymerase binds
- only 1 strand of the DNA is transcribed
- important site for regulation of gene expression

93

Global control by alternative σ-factors

***E. coli* σ-factors:**

σ^{70}	σ^{D}	*rpoD*	housekeeping sigma factor
σ^{32}	σ^{H}	*rpoH*	„heat shock" gene regulation
σ^{24}	σ^{E}	*rpoE*	envelope stress genes
σ^{38}	σ^{S}	*rpoS*	general stress response genes
σ^{28}	σ^{F}	*fliA*	flagellar chemotaxis genes
σ^{54}	σ^{N}	*rpoN*	nitrogen assimilation genes
σ...	σ^{FecI}	*fecI*	iron citrate uptake genes

General "stress response" sigma factor confers protection against:
- H_2O_2 and oxygen radicals
- acid and basic pH
- ethanol
- cold
- virulence factor control
- desiccation
- osmotic stress
- heat
- biofilm formation
- programmed cell death

- **and cross-protection!**

RpoS seems to be the "master regulator" for controlling a complex regulatory network.

94

Summary and future direction

Introduction and overview light exposure systems

Importance of cellular state

Importance of multi-parameter assessment and use of flow cytometry

Summary & outlook

A range of methods is now established in the lab for identifying the damage at the single cell level (many of them based on flow cytometry)

The "agony" of *E. coli* during solar stress has been demonstrated to start at the cytoplasmic membrane level and the energy status

A dose of 1500 kJ m^2 solar UVA (corresponding to 530 W m^2 global sunlight intensity for 6 h, which is reached in most areas easily) is sufficient for safe inactivation of enterobacteria

and for the future…

Confirming the sequence of damage seen in *E. coli* for pathogens (*Vibrio cholerae, Salmonella, Shigella* strains)

Identifying the major mechanism of damage at the molecular level in the different phases, confirm that it is identical in the different pathogenic enterobacteria

95

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and for the future… (some examples)

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96

Indications in the literature:

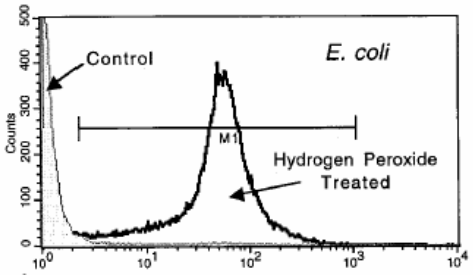
microbial resistance to disinfectants and stress can depend on growth rate

97

Detecting damage: DNA

„Any damage" to DNA (other than T-T dimers) that is repaired results in ss-DNA with free 3'-OH ends that serve as free ends for new DNA synthesis. These ends are enzymatically labelled with dUTP-fluorescein isothiocyanate.

Terminal desoxyribonucleotide transferase mediated dUTP nick end labelling (TUNEL) method used routinely for apoptosis detection. It has been applied to bacteria (Rhower & Azam, 2000, AEM 66:1001) and measured by flow cytometry.

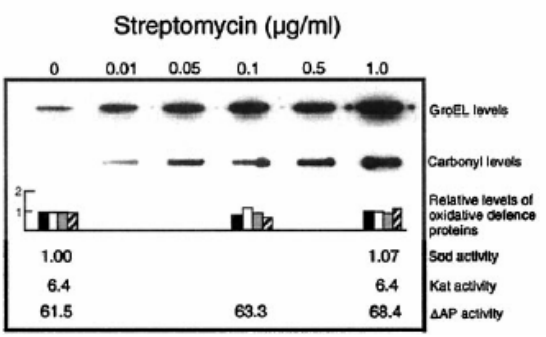


98

Detecting damage: protein

Oxidative protein damage results in carbonylation of some amino acids (-C=O).

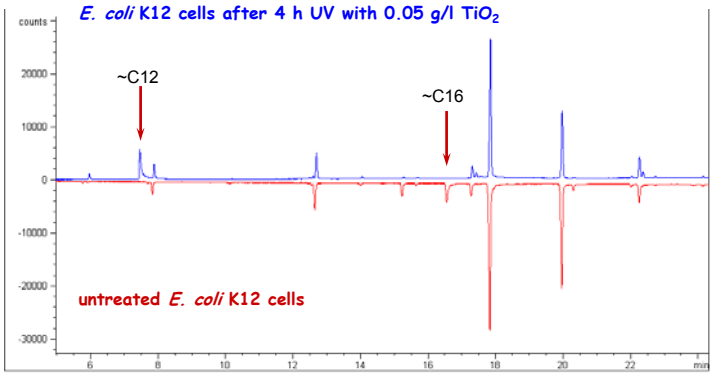
Carbonylation assays: R-C=O side chains are drivatized with commercial kit to 2,4-dinitrophenyl hydrazones and then detected using 2,4-DNPH-specific antibodies.



Exposure of *E. coli* to streptomycin. From Dukan et al. (2000) PNAS, 97:5746

Detecting damage: membrane

- Lipid analysis (FAME), e.g., epoxide formation at unsaturated fatty acids)



(data from Gottfried Dasen)

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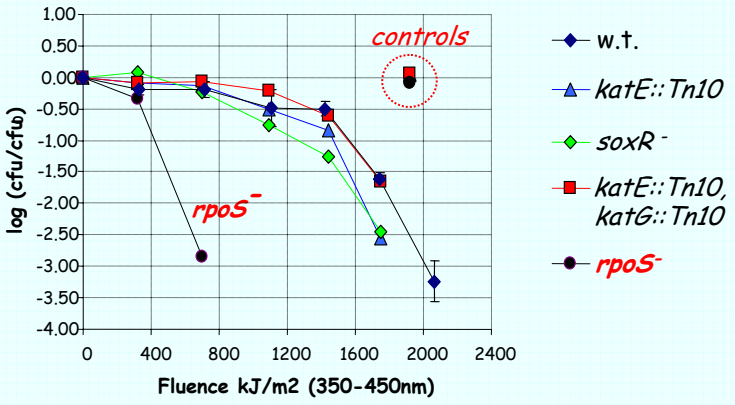
Survival of different *E. coli* mutants in sunlight

(sensitive to reactive oxygen species or general stress response)



From Berney *et al.* (2006) AEM 72, 2586-93