Microbial inactivation by the solar-assisted Fenton process at near-neutral pH

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Introduction? Lucky to present so late ☺

From Fenton…

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-}$$

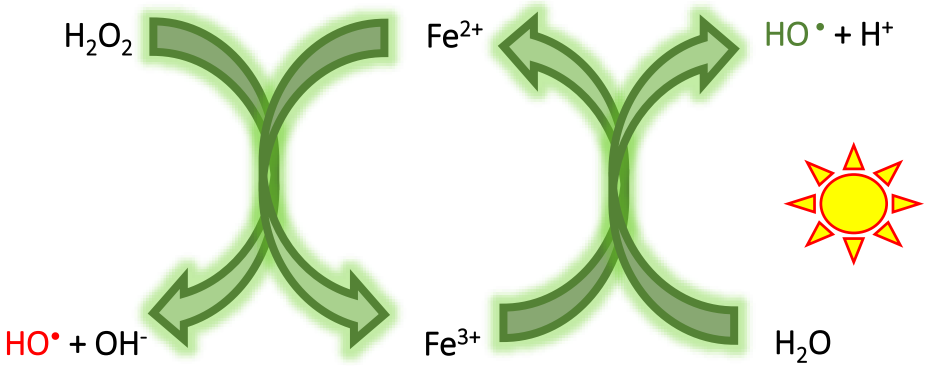
k_1=76 $M^{-1}s^{-1}$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^{+}$$

k_2=0.01 $M^{-1}s^{-1}$

Limiting step!

…to photo-Fenton



$$Fe^{3+} + H_2O \xrightarrow{hv} Fe^{2+} + H^{+} + HO^{\bullet}$$

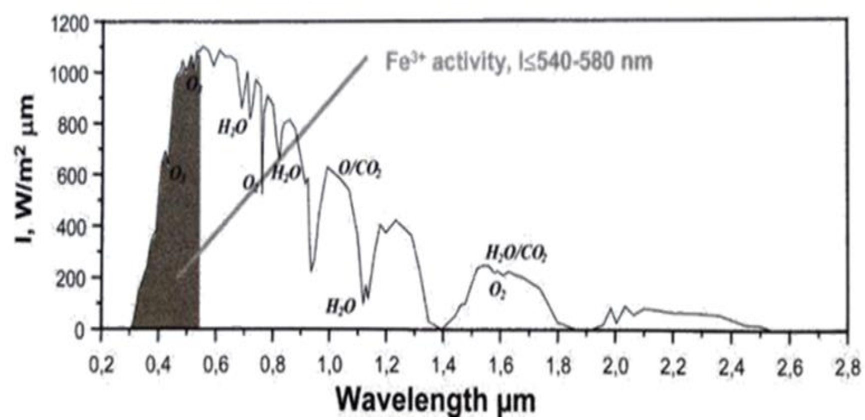


Photo-catalytic significance of iron

Reaction No.	*Reaction*	*Reaction Constant*
(1)	$Fe^{3+} + H_2O \leftrightarrow \mathrm{Fe(OH)}^{2+} + H^+$	$(k_1 = 2.9x10^{-3}M)$
(2)	$Fe^{3+} + 2H_2O \leftrightarrow \mathrm{Fe(OH)}_2^+ + 2H^+$	$(k_2 = 7.62x10^{-7}M^2)$
(3)	$2Fe^{3+} + 2H_2O \leftrightarrow \mathrm{Fe_2(OH)}_2^{4+} + 2H^+$	$(k_{2.2} = 0.8x10^{-3}M)$
(4)	$Fe^{3+} + H_2O_2 \leftrightarrow \mathrm{Fe}^{3+}(\mathrm{HO_2})^{2+} + H^+$	$(kI_1 = 3.1x10^{-3})$
(5)	$\mathrm{Fe(OH)}^{2+} + H_2O_2 \leftrightarrow \mathrm{Fe}^{3+}(OH)(\mathrm{HO_2})^+ + H^+$	$(kI_2 = 2x10^{-4})$
(6a)	$\mathrm{Fe}^{3+}(\mathrm{HO_2})^{2+} \rightarrow \mathrm{Fe}^{2+} + HO_2^\bullet$	$(k_6 = x10^{-3}s^{-1})$
(6b)	$\mathrm{Fe}^{3+}(OH)(\mathrm{HO_2})^+ \rightarrow \mathrm{Fe}^{2+} + HO_2^\bullet + OH^-$	$(k_6 = x10^{-3}s^{-1})$
(7)	$\mathrm{Fe}^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^\bullet + OH^-$	$(k_7 = 63\ M^{-1}s^{-1})$
(8)	$\mathrm{Fe}^{2+} + HO^\bullet \rightarrow Fe^{3+} + OH^-$	$(k_8 = 3.2x10^8M^{-1}s^{-1})$
(9)	$HO^\bullet + H_2O_2 \rightarrow HO_2^\bullet + H_2O$	$(k_9 = 3.3x10^9M^{-1}s^{-1})$
(10a)	$\mathrm{Fe}^{2+} + HO_2^\bullet \rightarrow \mathrm{Fe}^{3+}(\mathrm{HO_2})^{2+}$	$(k_{10a} = 1.2x10^6M^{-1}s^{-1})$
(10b)	$\mathrm{Fe}^{2+} + O_2^{\bullet-} + H^+ \rightarrow \mathrm{Fe}^{3+}(\mathrm{HO_2})^{2+}$	$(k_{10b} = 1x10^7M^{-1}s^{-1})$
(11a)	$\mathrm{Fe}^{3+} + HO_2^\bullet \rightarrow \mathrm{Fe}^{2+} + O_2 + H^+$	$(k_{11a} < 2x10^3M^{-1}s^{-1})$
(11b)	$\mathrm{Fe}^{3+} + O_2^{\bullet-} \rightarrow \mathrm{Fe}^{2+} + O_2$	$(k_{11b} = 5x10^7M^{-1}s^{-1})$
(12a)	$HO_2^\bullet \rightarrow O_2^{\bullet-} + H^+$	$(k_{12a} = 1.58x10^5M^{-1}s^{-1})$
(12b)	$O_2^{\bullet-} + H^+ \rightarrow HO_2^\bullet$	$(k_{12b} = 1x10^{10}M^{-1}s^{-1})$
(13a)	$HO_2^\bullet + HO_2^\bullet \rightarrow H_2O_2 + O_2$	$(k_{13\mathrm{a}} = 8.3x10^5M^{-1}s^{-1})$
(13b)	$HO_2^\bullet + O_2^{\bullet-} + H_2O \rightarrow H_2O_2 + O_2 + OH^-$	$(k_{13\mathrm{b}} = 9.7x10^7M^{-1}s^{-1})$
(14a)	$HO^\bullet + HO_2^\bullet \rightarrow H_2O + O_2$	$(k_{14\mathrm{a}} = 0.71x10^{10}M^{-1}s^{-1})$
(14b)	$HO^\bullet + O_2^{\bullet-} \rightarrow O_2 + OH^-$	$(k_{14\mathrm{b}} = 1.01x10^{10}M^{-1}s^{-1})$
(15)	$HO^\bullet + HO^\bullet \rightarrow H_2O_2$	$(k_{15} = 5.2x10^9M^{-1}s^{-1})$

Reactions initiated by iron

Initiation
Propagation
Termination

De Laat, J., & Gallard, H. (1999). Catalytic decomposition of hydrogen peroxide by Fe (III) in homogeneous aqueous solution: mechanism and kinetic modeling. Environmental Science & Technology, 33(16), 2726-2732.

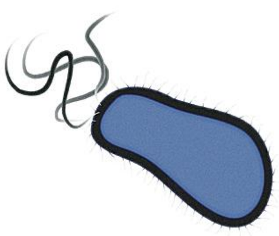
Highlights of the present work

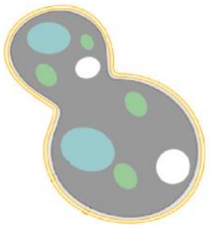
Microorganism inactivation

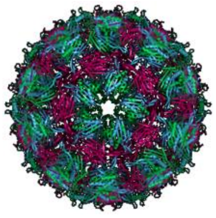
Kinetics of single-target elimination
- *Bacteria*
- *Viruses*
- *Yeasts*

Photo-Fenton: near-neutral pH
- *Low Fe(II),(III) and H_2O_2 concentration*
- *Controlled, simulated wastewater experiments*
- *Solar simulators as light source*

Bacteria



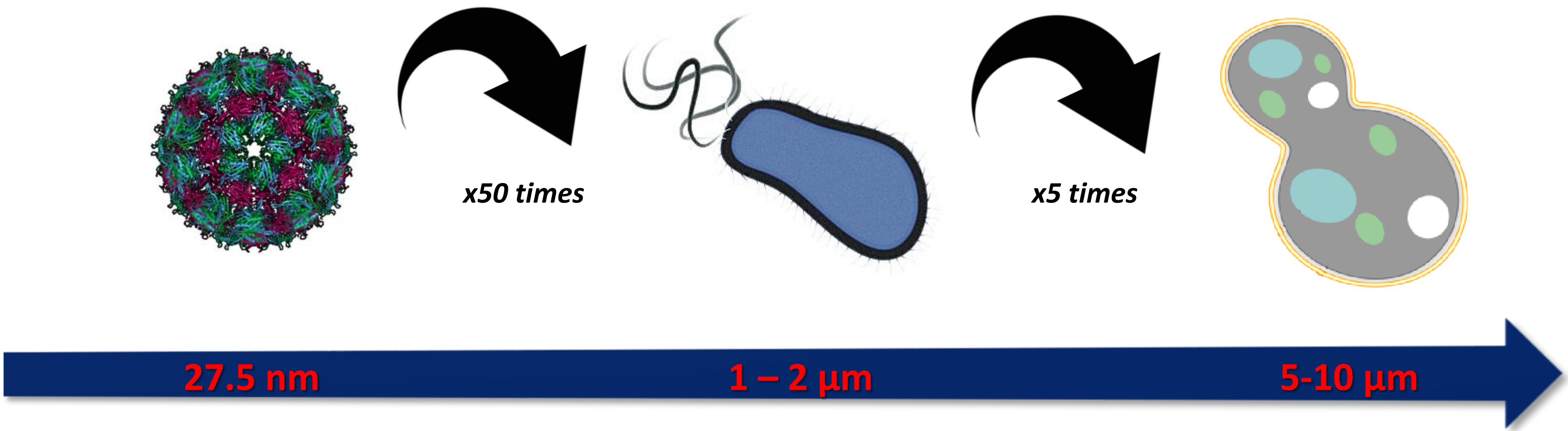




Yeasts **Viruses**

Our models…

Male Somatic 2 Coliphage | ***Escherichia coli* K-12** | ***Saccharomyces Cerevisiae***



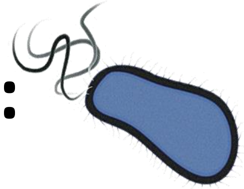
Structural differences

Surface coat protein
Packed under pressure

Only 1 to 2 layers of peptidoglycan
Plasma membrane

Chitin, thick outer layer
Double layer plasma membrane

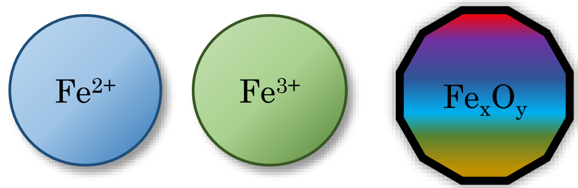
Storyline Guide:



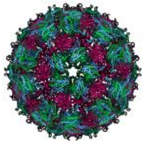
1) Action of solar light: baseline, and an AOP in disguise

2) Addition of H_2O_2

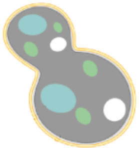
3) Addition of Iron



4) Viruses – what changes?



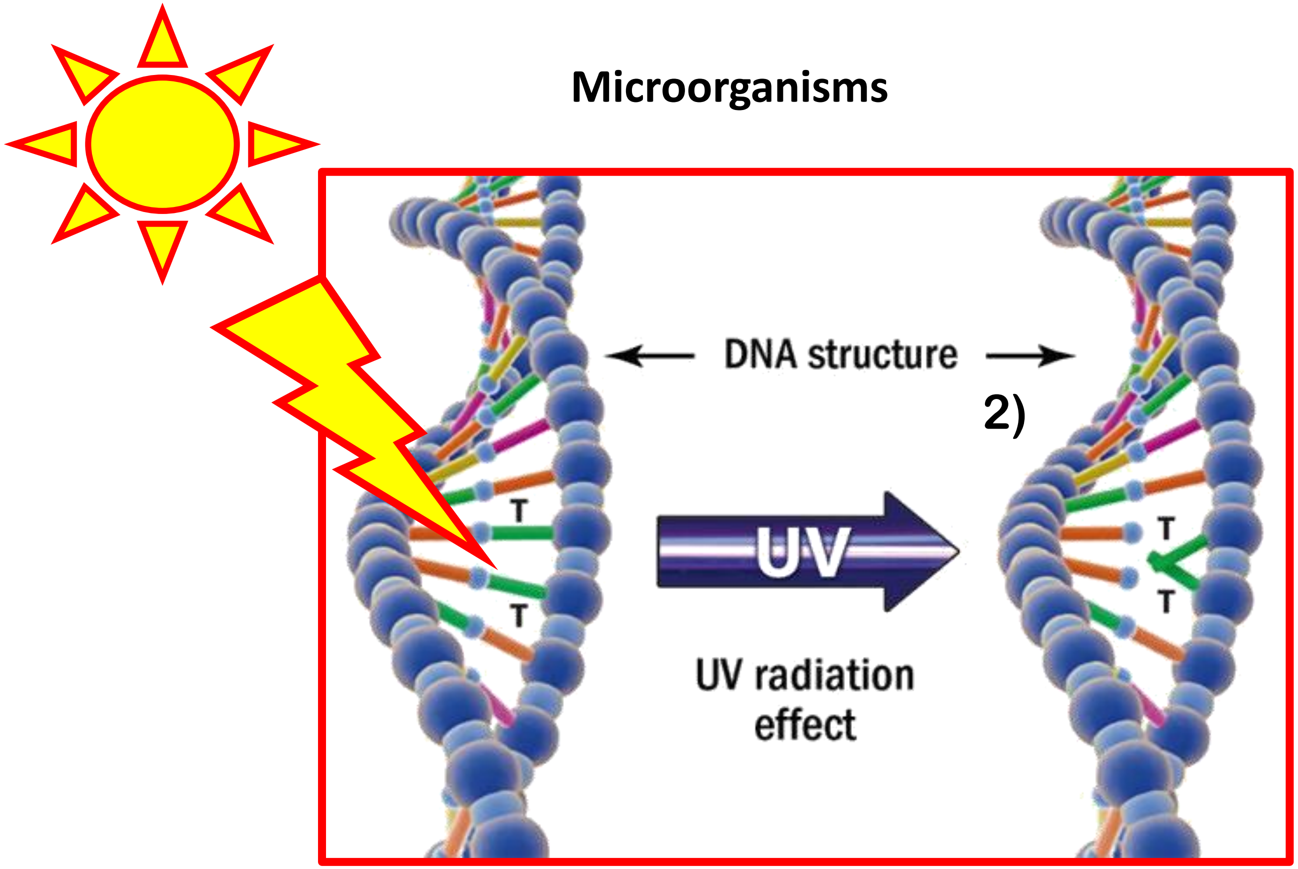
5) Yeasts – Similarities and differences



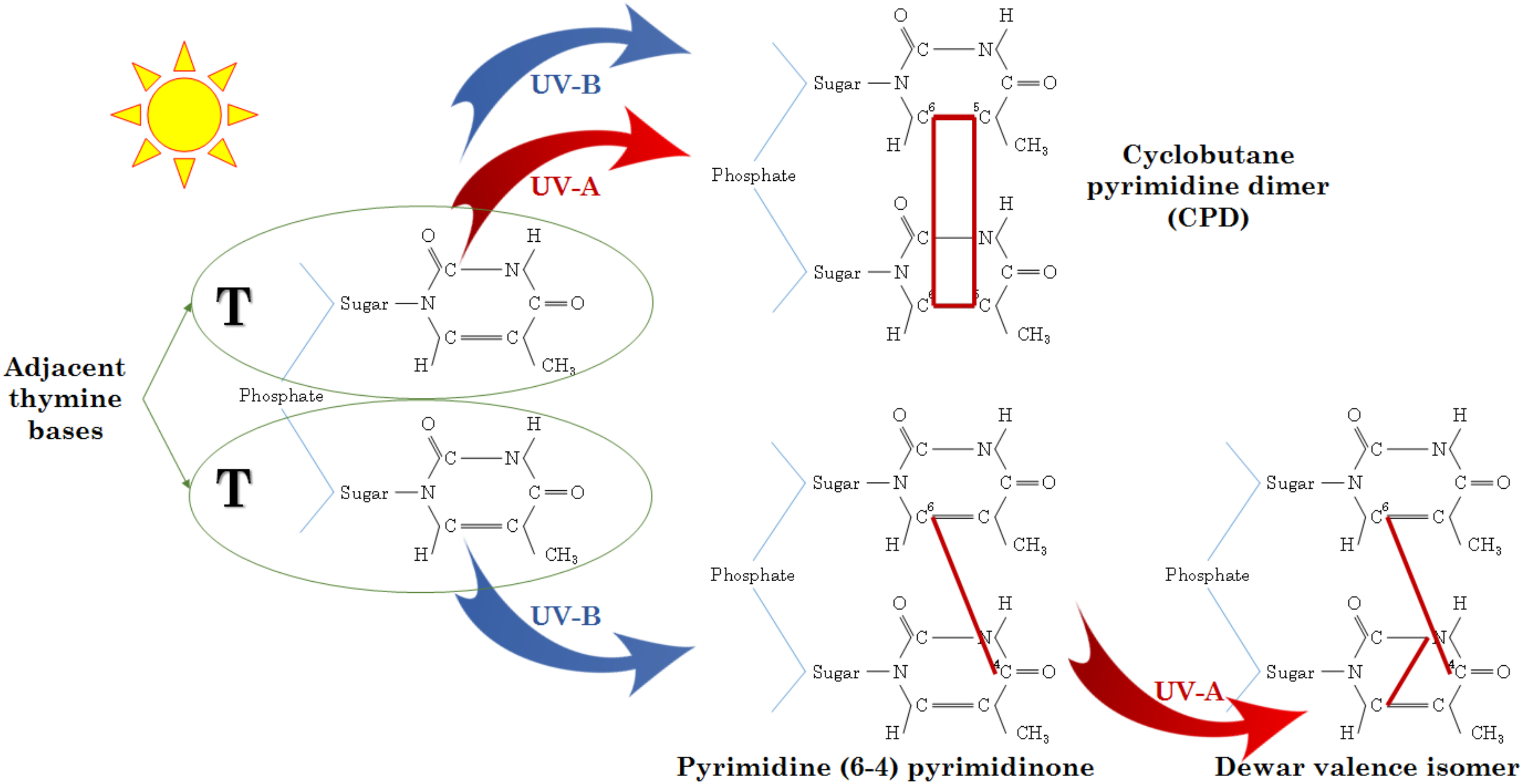
6) Effect of the matrix

Bacterial inactivation:
Step-wise construction of a mechanistic interpretation

Baseline: effect of solar light



Direct action of light

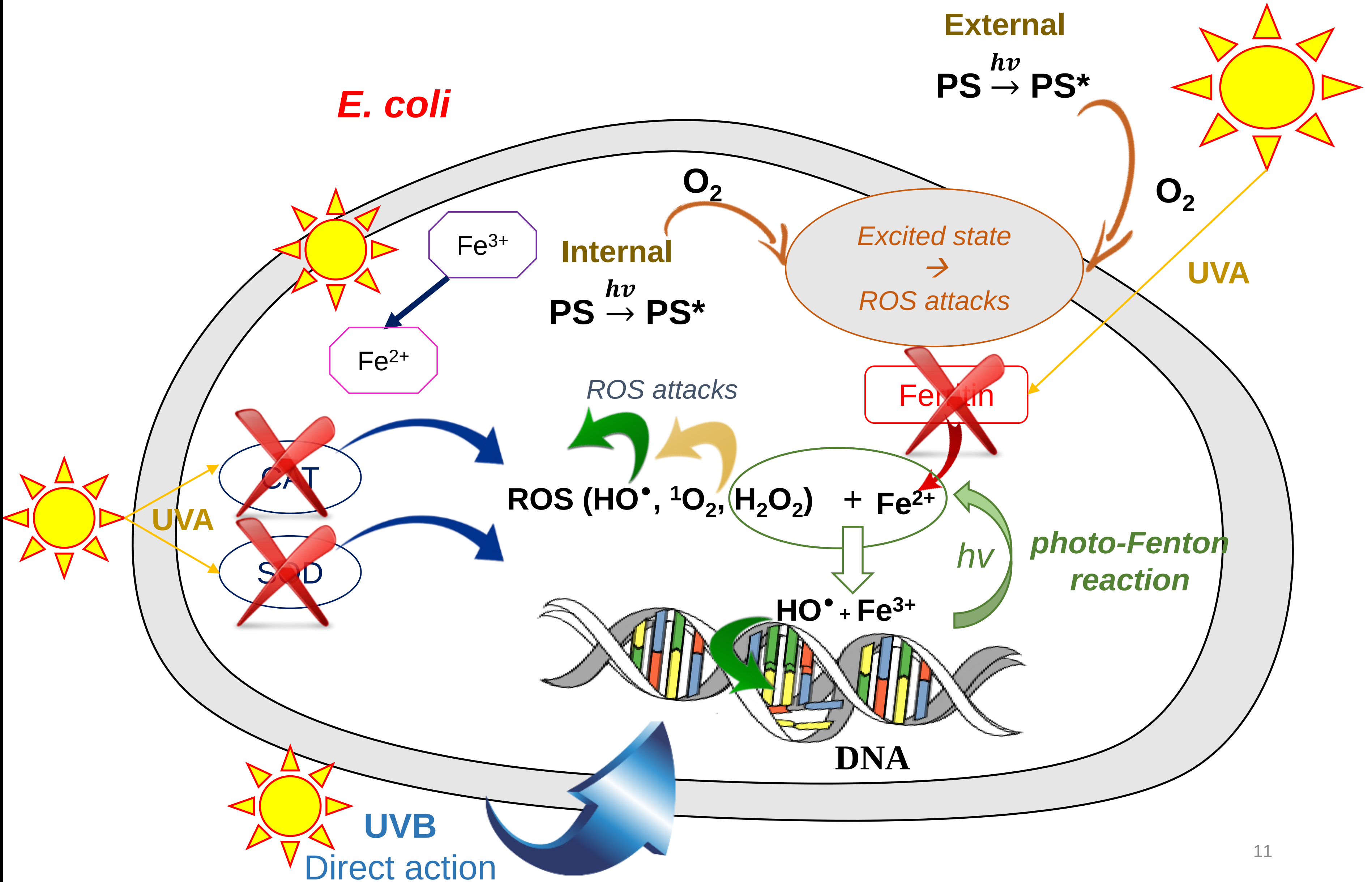


Repair!

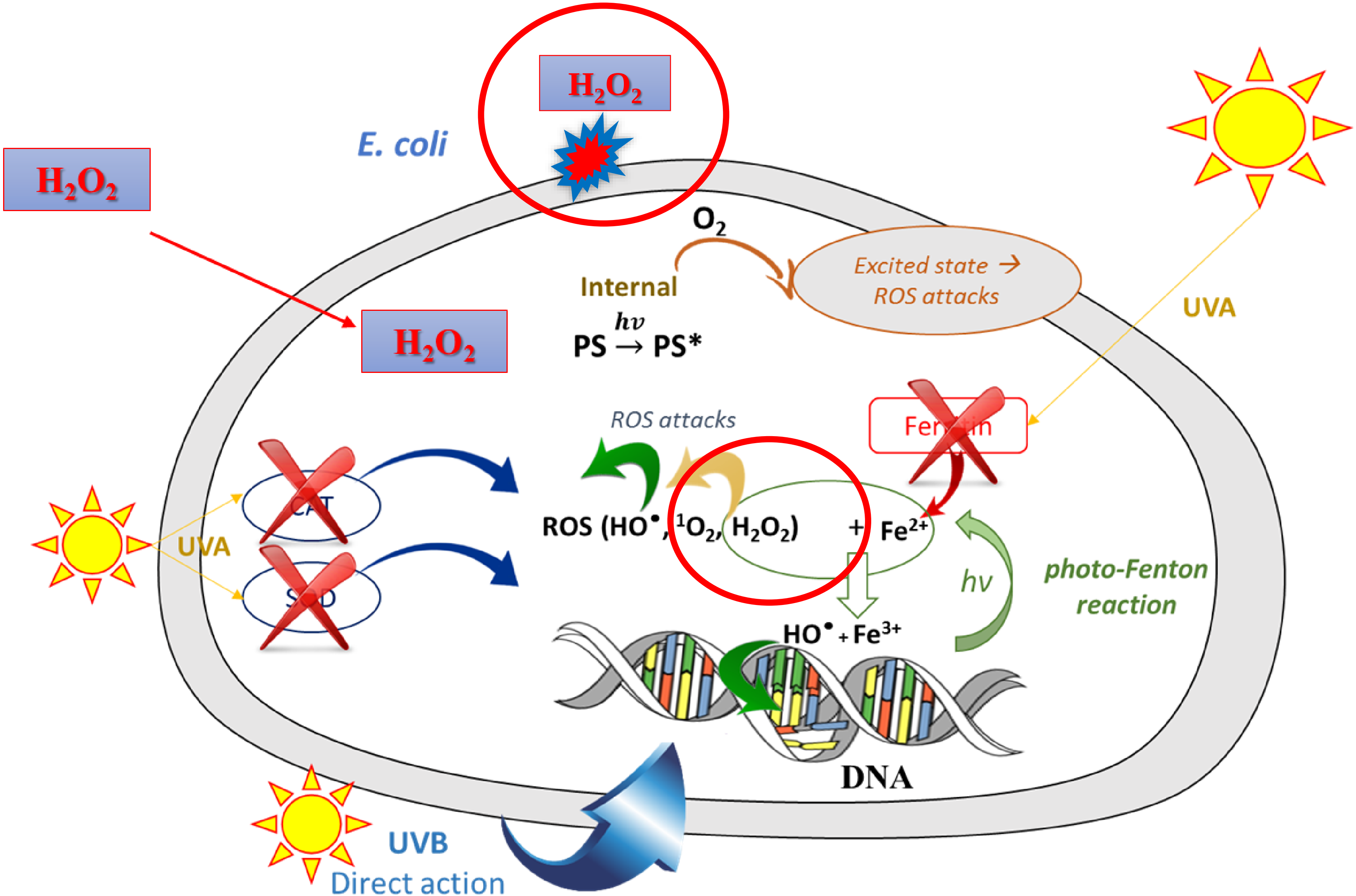
Solar light alone (?)

Solar light alone is an
“indirect” AOP

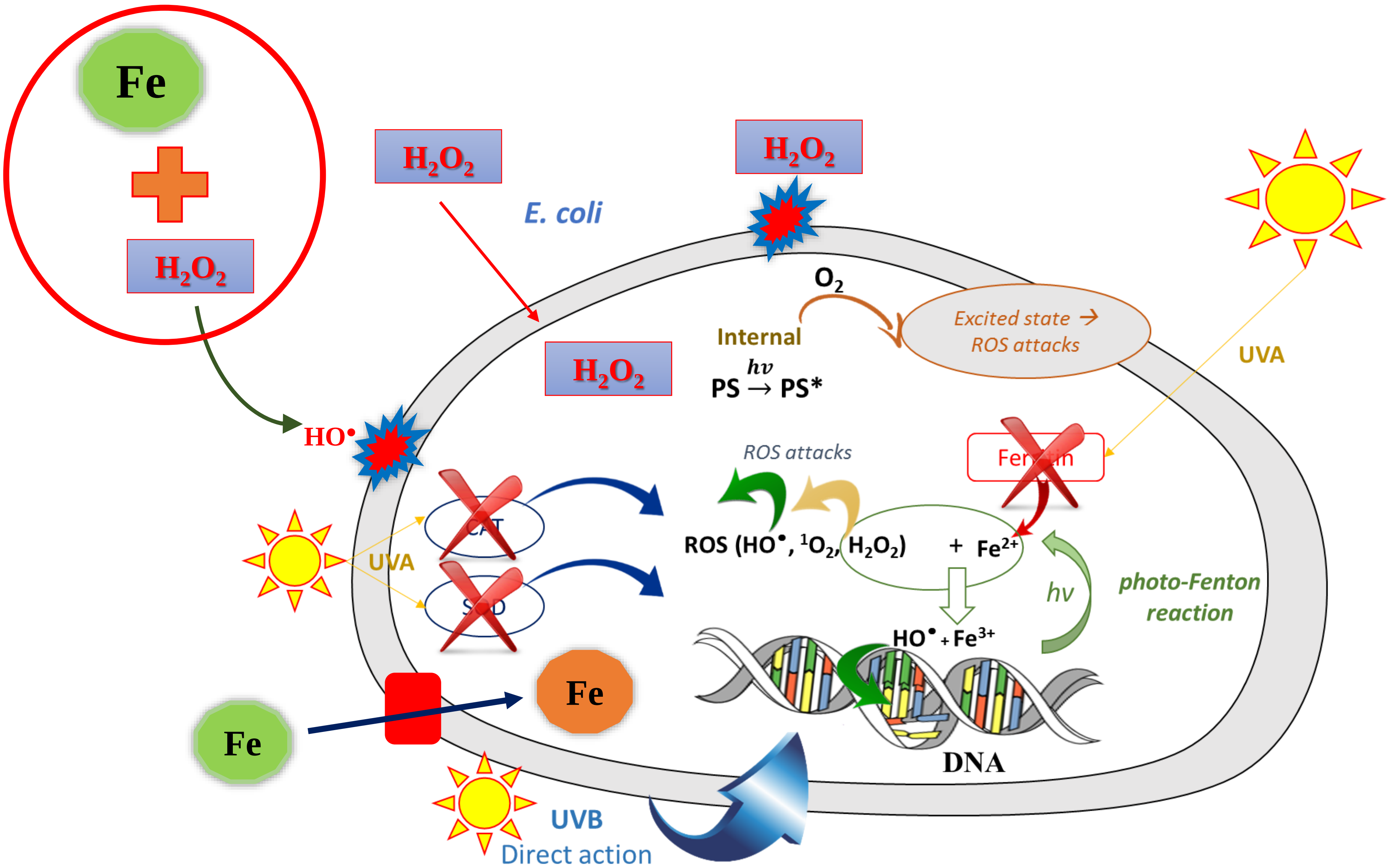
ONLY BY SOLAR LIGHT



Solar light + H_2O_2



Solar light + H_2O_2 + Fe



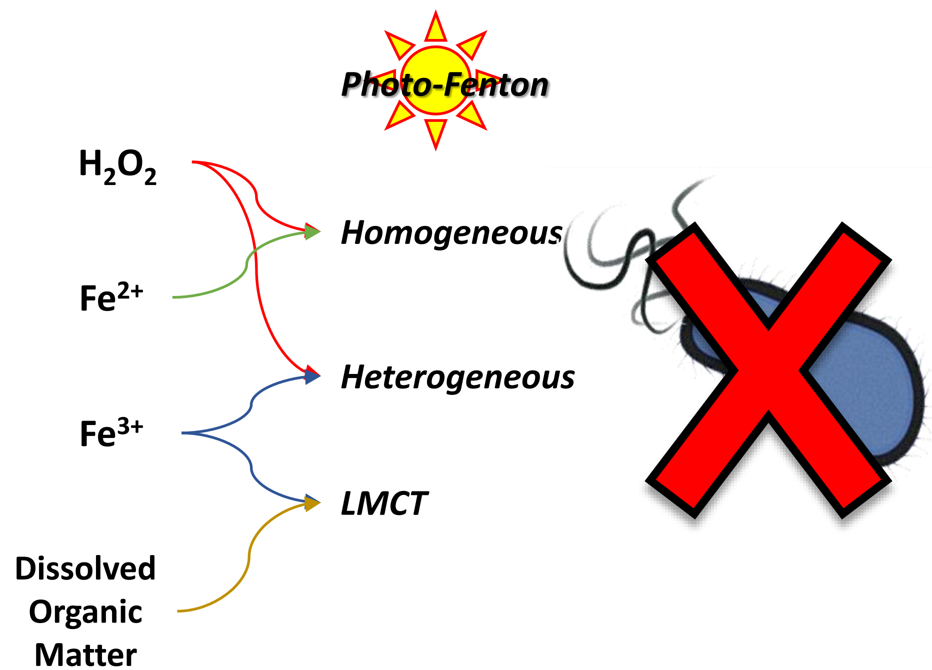
Previous work on bacteria

Applied Catalysis B: Environmental
Volume 96, Issues 1–2, 26 April 2010, Pages 126–141

The effect of Fe^{2+}, Fe^{3+}, H_2O_2 and the photo-Fenton reagent at near neutral pH on the solar disinfection (SODIS) at low temperatures of water containing *Escherichia coli* K12

Dorothee Spuhler, Julian Andrés Rengifo-Herrera, César Pulgarin
Institute of Chemical Sciences and Engineering (ISIC), EPF Lausanne, CH-1015 Lausanne, Switzerland

Received 26 November 2009, Revised 1 February 2010, Accepted 4 February 2010, Available online 12 February 2010



But also...

Ruales-Lonfat, C., Benítez, N., Sienkiewicz, A. and Pulgarín, C. (2014)
Deleterious effect of homogeneous and heterogeneous near-neutral photo-Fenton system on Escherichia coli. Comparison with photo-catalytic action of TiO_2 during cell envelope disruption. ***Applied Catalysis B: Environmental 160, 286-297.***

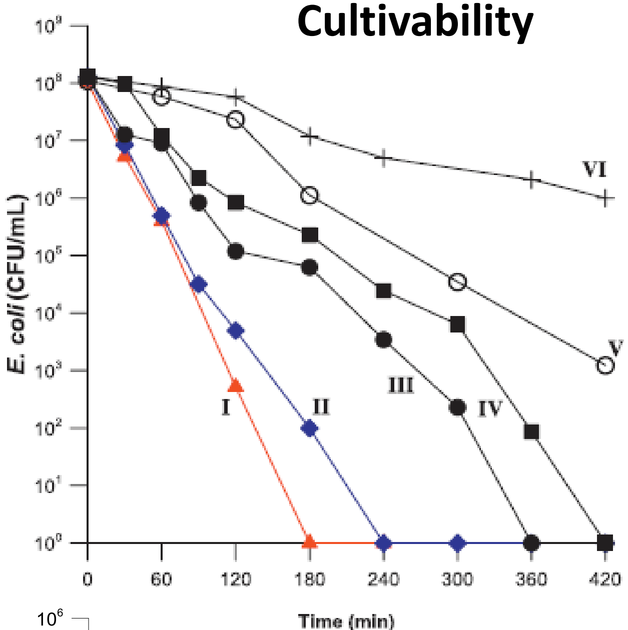
Ndounla, J., Kenfack, S., Wéthé, J. and Pulgarin, C. (2014)
Relevant impact of irradiance (vs. dose) and evolution of pH and mineral nitrogen compounds during natural water disinfection by photo-Fenton in a solar CPC reactor. ***Applied Catalysis B: Environmental 148-149, 144-153.***

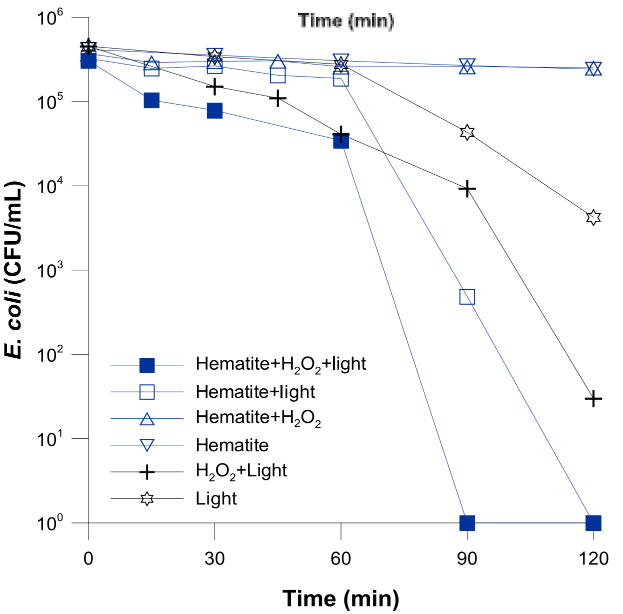
Ruales-Lonfat, C., Barona, J. F., Sienkiewicz, A., Bensimon, M., Vélez-Colmenares, J., Benítez, N., & Pulgarín, C. (2015).
Iron oxides semiconductors are efficients for solar water disinfection: A comparison with photo-Fenton processes at neutral pH.
***Applied Catalysis B: Environmental*, *166*, 497-508.**

...and many more

Iron oxides as semiconductors and as pF catalysts

Cultivability





Results:

I: TiO_2 photocatalysis

II: pF with $FeSO_4$

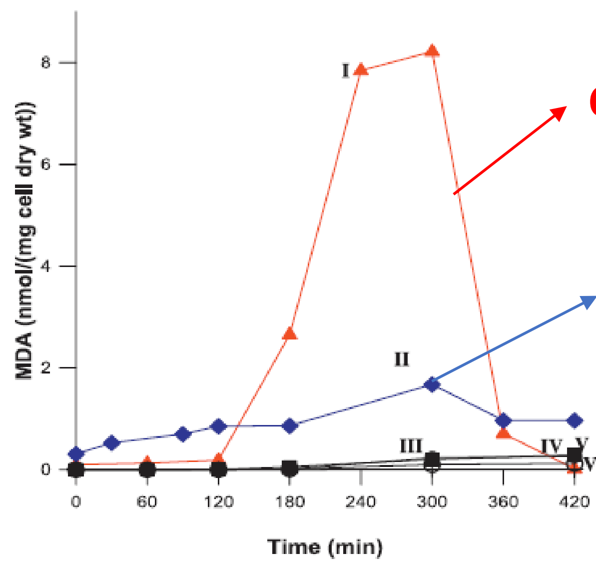
III: hv/$FeSO_4$

IV: hv/H_2O_2

V: Solar light

VI: Fenton (dark)

MDA formation

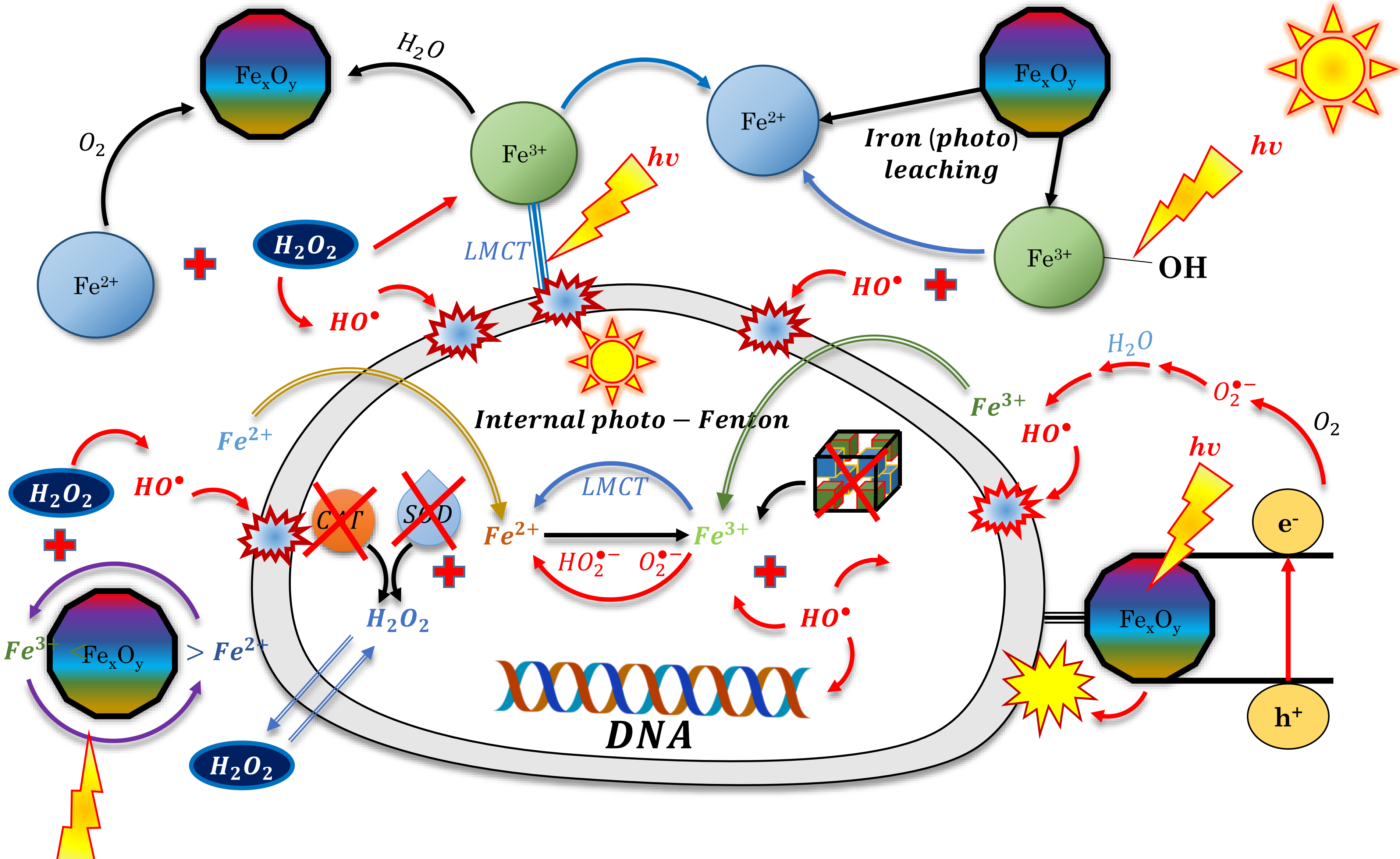


Cell wall degradation

Internal damage

Ruales-Lonfat, C., Benítez, N., Sienkiewicz, A., & Pulgarín, C. (2014).. Applied Catalysis B: Environmental, 160, 286-297.

Integrated mechanism



Giannakis, *S., Polo López, M.I., Spuhler, D., Sánchez Pérez, J.A., Fernández Ibáñez, P., Pulgarin, C. (2016) Applied Catalysis B: Environmental, 199, pp. 199-223.*
Giannakis, *S., Polo López, M.I., Spuhler, D., Sanchez Pérez, J.A., Fernandez Ibáñez, P., Pulgarin, C. (2016) Applied Catalysis B: Environmental, 198, pp. 431-446.*

Previous work on viruses

Applied Catalysis B: Environmental

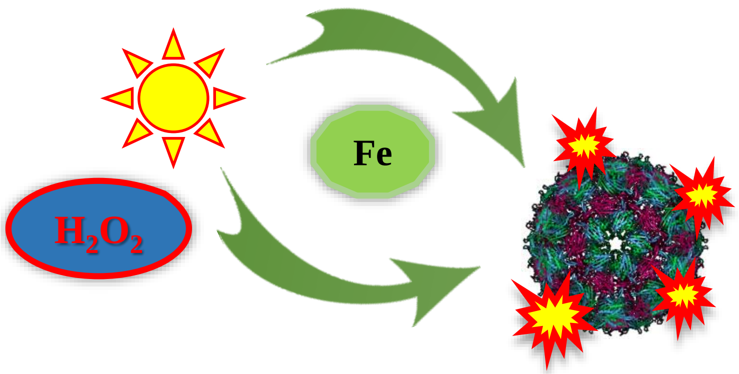
Volumes 174–175, September 2015, Pages 395–402

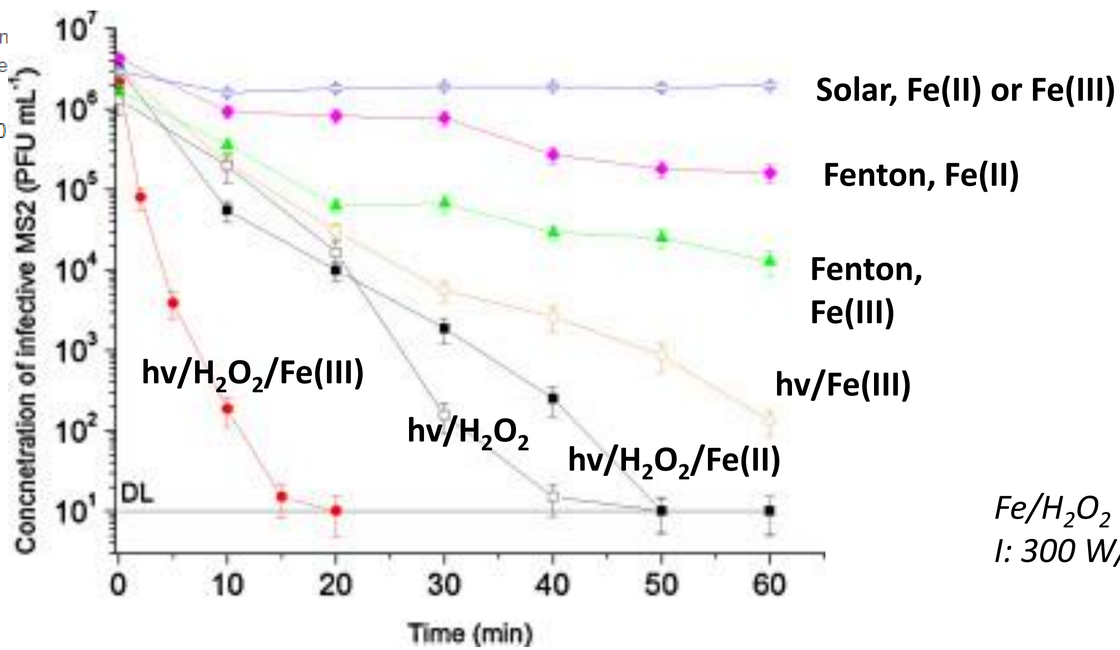
Principal parameters affecting virus inactivation by the solar photo-Fenton process at neutral pH and μM concentrations of H_2O_2 and $Fe^{2+/3+}$

E. Ortega-Gómez[a, b], M.M. Ballesteros Martín[b, d], A. Carratalà[c], P. Fernández Ibañez[b, e], J.A. Sánchez Pérez[a, b], C. Pulgarín[f]

[a] Department of Chemical Engineering, University of Almería, 04120 Almería, Spain
[b] CIESOL, Joint Centre of the University of Almería-CIEMAT, 04120 Almería, Spain
[c] Laboratory of Environmental Chemistry, School of Architecture, Civil and Environmental Engineering (ENAC), École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[d] Department of Molecular Biology and Biochemical Engineering, University of Pablo de Olavide, 41013 Sevilla, Spain
[e] Plataforma Solar de Almería, CIEMAT, 04200 Tabern
[f] École Polytechnique Fédérale de Lausanne, Institute
Station 6, CH-1015 Lausanne, Switzerland

Received 13 November 2014, Revised 11 February 20
March 2015

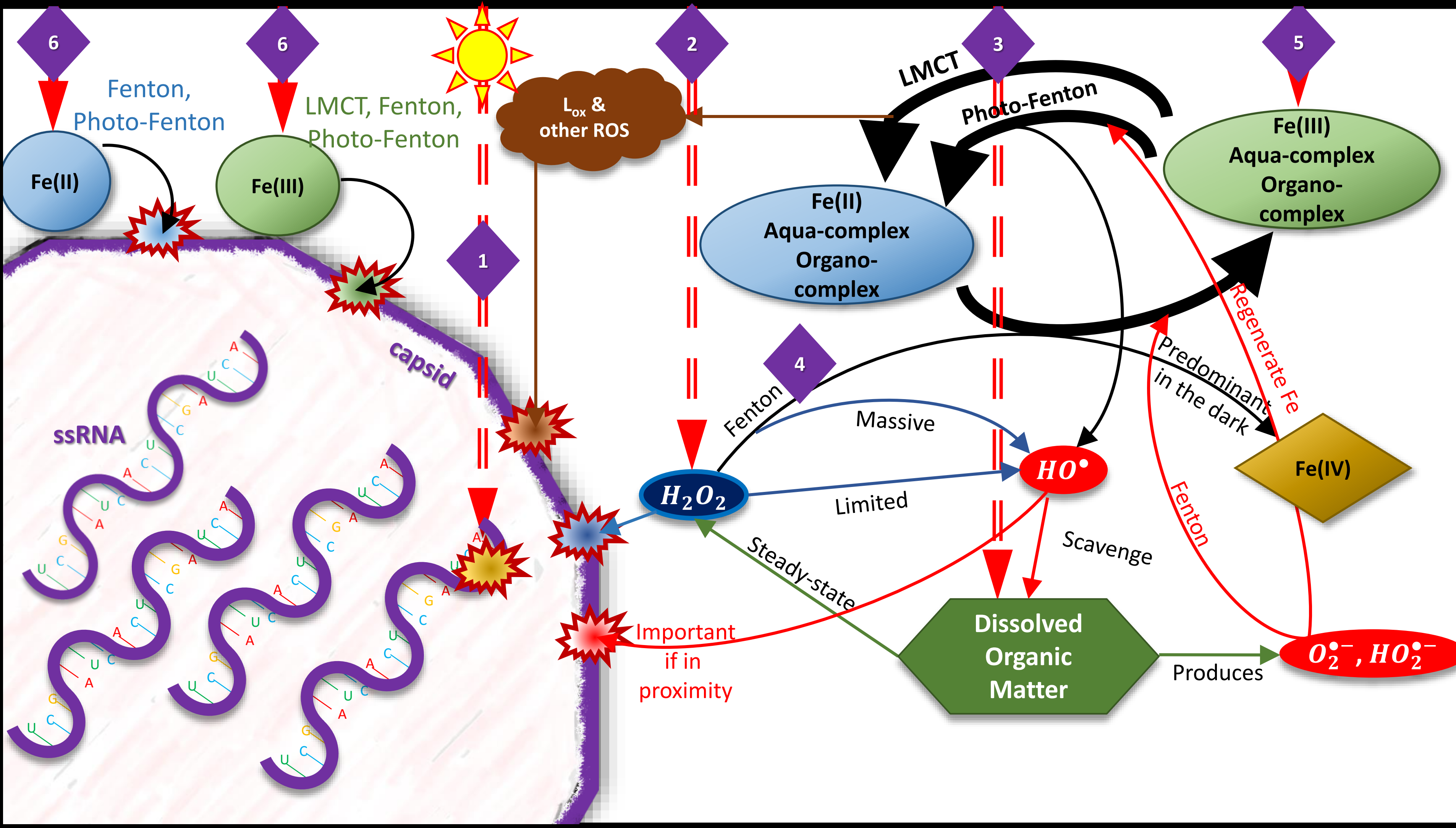




Fe/H_2O_2 ratio: 1/1
I: 300 W/m^2

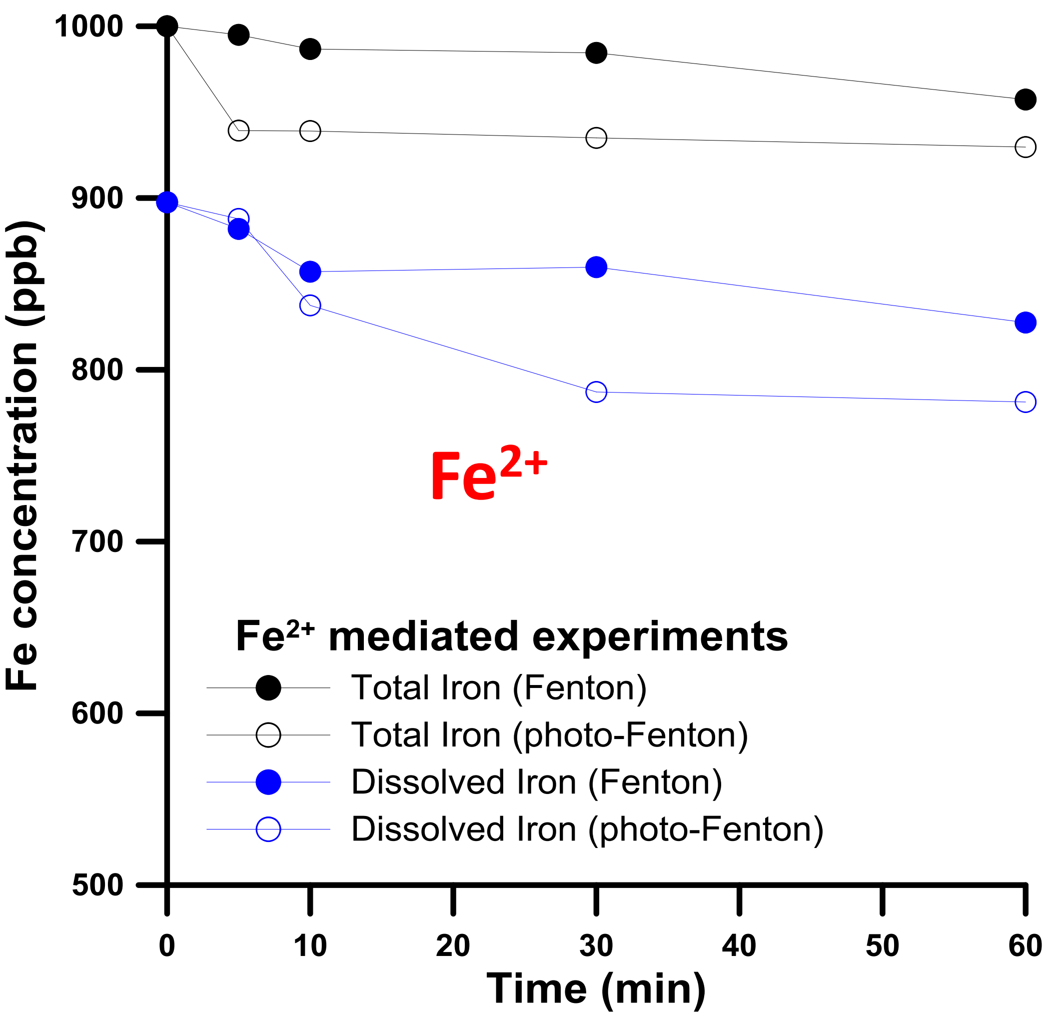
Ortega-Gomez et al. (2015), App. Cat. B., pp. 395-402

Proposed inactivation mechanism

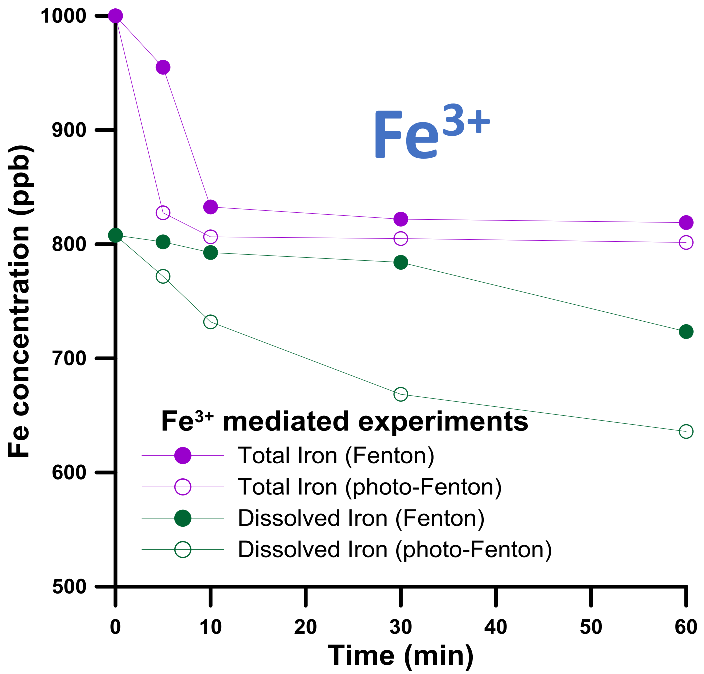


S. Giannakis, S. Liu, A. Carratala, S. Rtimi, M. Bensimon, C. Pulgarin, Applied Catalysis B: Environmental (2017).

Key to inactivation: iron complexation with HA



ICP-MS results

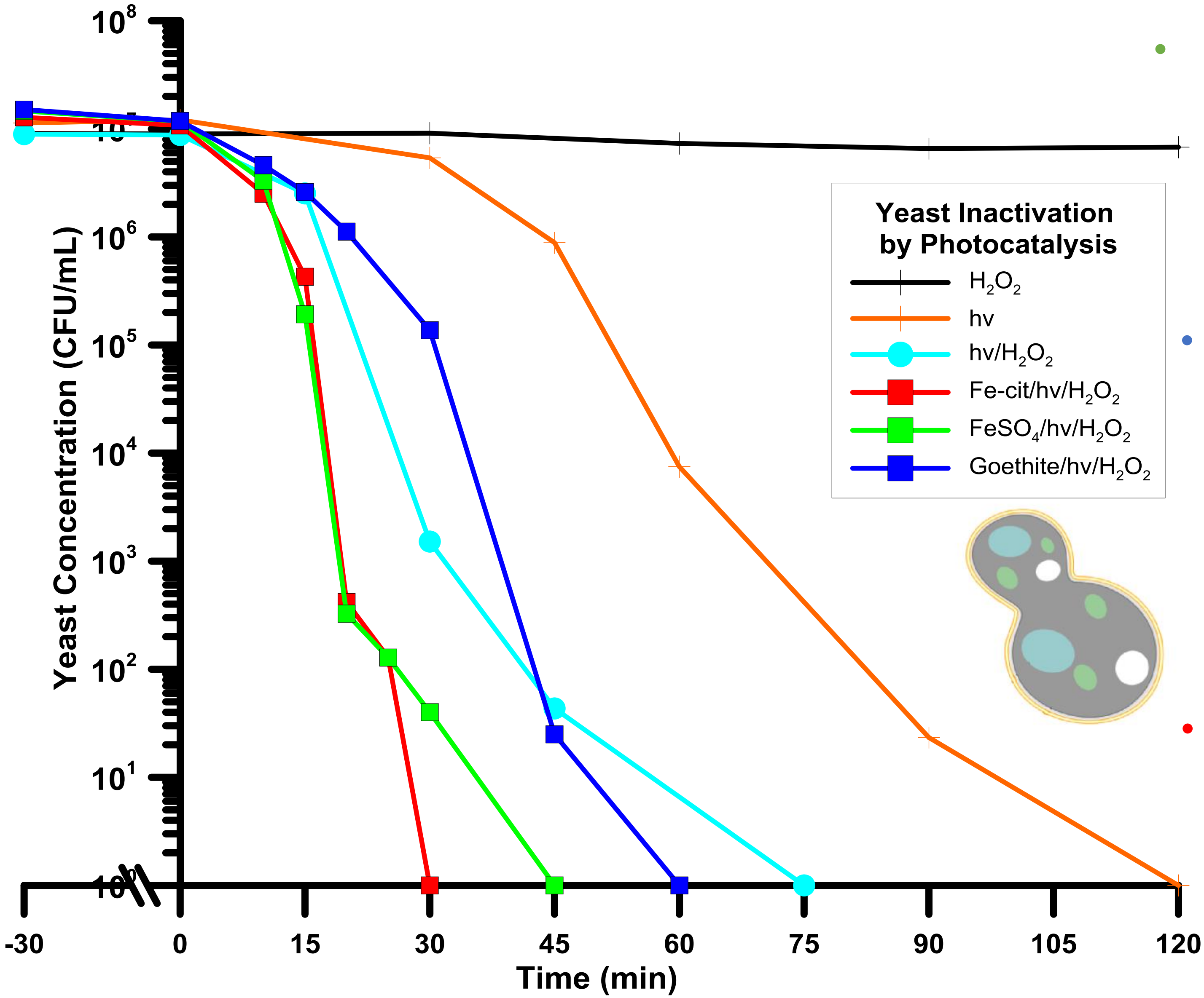


Available iron throughout the test !

Photo-Fenton takes place!

S. Giannakis, S. Liu, A. Carratala, S. Rtimi, M. Bensimon, C. Pulgarin, Applied Catalysis B: Environmental (2017).

Yeast Inactivation: a brief summary in MQ



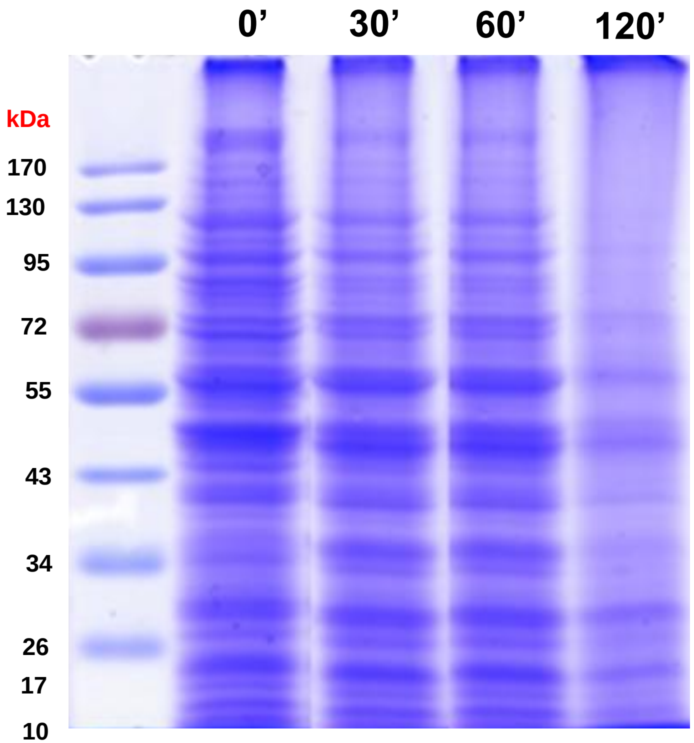
- **$hv/H_2O_2/FeSO_4$**: mainly homogeneous external process
- **$hv/H_2O_2/Fe$-Oxides**: **goethite-natural** semiconductor or heterogeneous mode of action
- **$hv/H_2O_2/Fe$-Citrate**: **mild complex** achieving homogeneous process in higher pH

S. Giannakis, C. Ruales-Lonfat, S. Rtimi, S. Thabet, P. Cotton, C. Pulgarin, Applied Catalysis B: Environmental 185 (2016) 150-162.

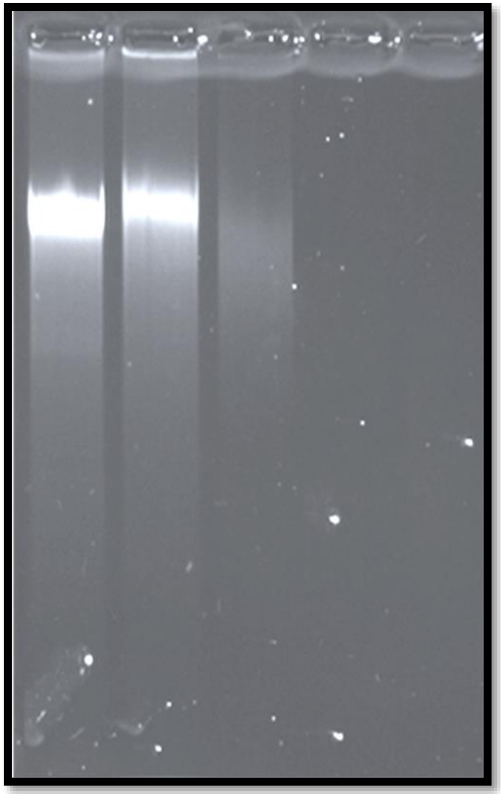
DNA and protein damages (photo-Fenton process)

$h\nu/FeSO_4/H_2O_2$ at pH = 5.5

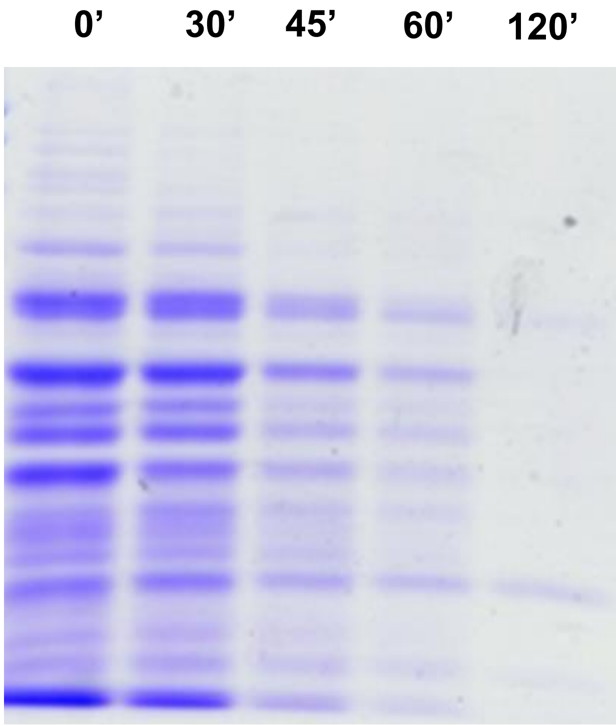
Cell wall proteins



DNA

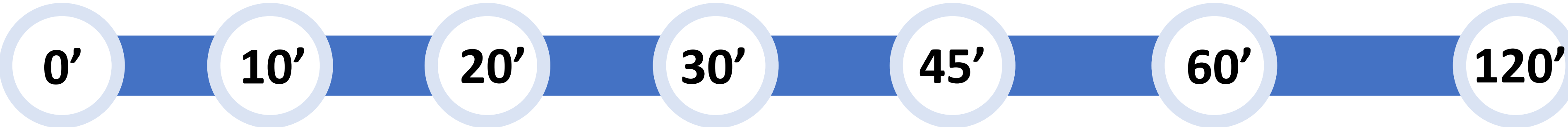


Cytoplasmic proteins



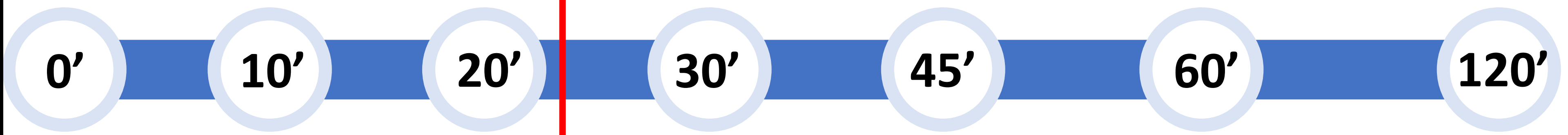
S. Giannakis, C. Ruales-Lonfat, S. Rtimi, S. Thabet, P. Cotton, C. Pulgarin, Applied Catalysis B: Environmental 185 (2016) 150-162.

Establishing a timeline for hv/H_2O_2/Fe, verifies that…

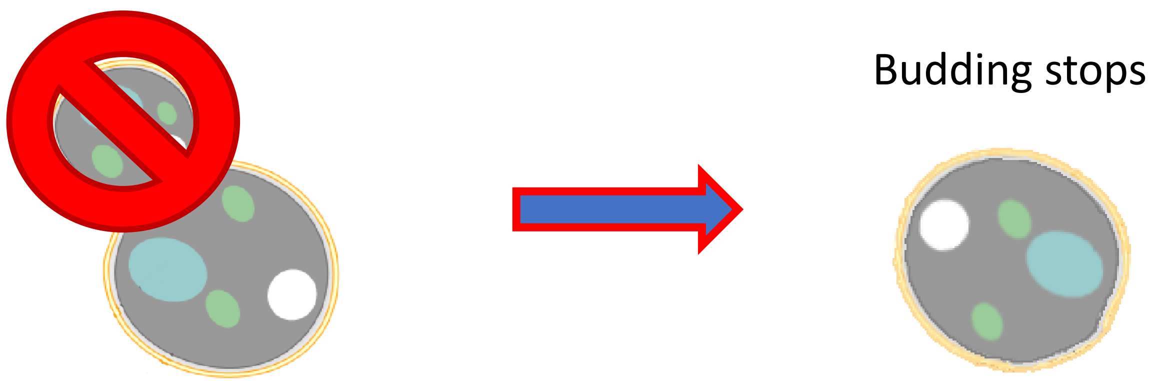


…internal photo-Fenton is the driving inactivation force!

Establishing a timeline for hv/H_2O_2/Fe, verifies that…



Loss of cultivability: 1st indication of inactivation



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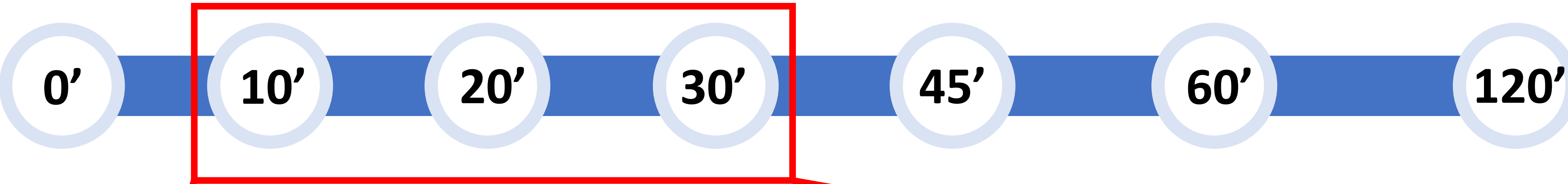
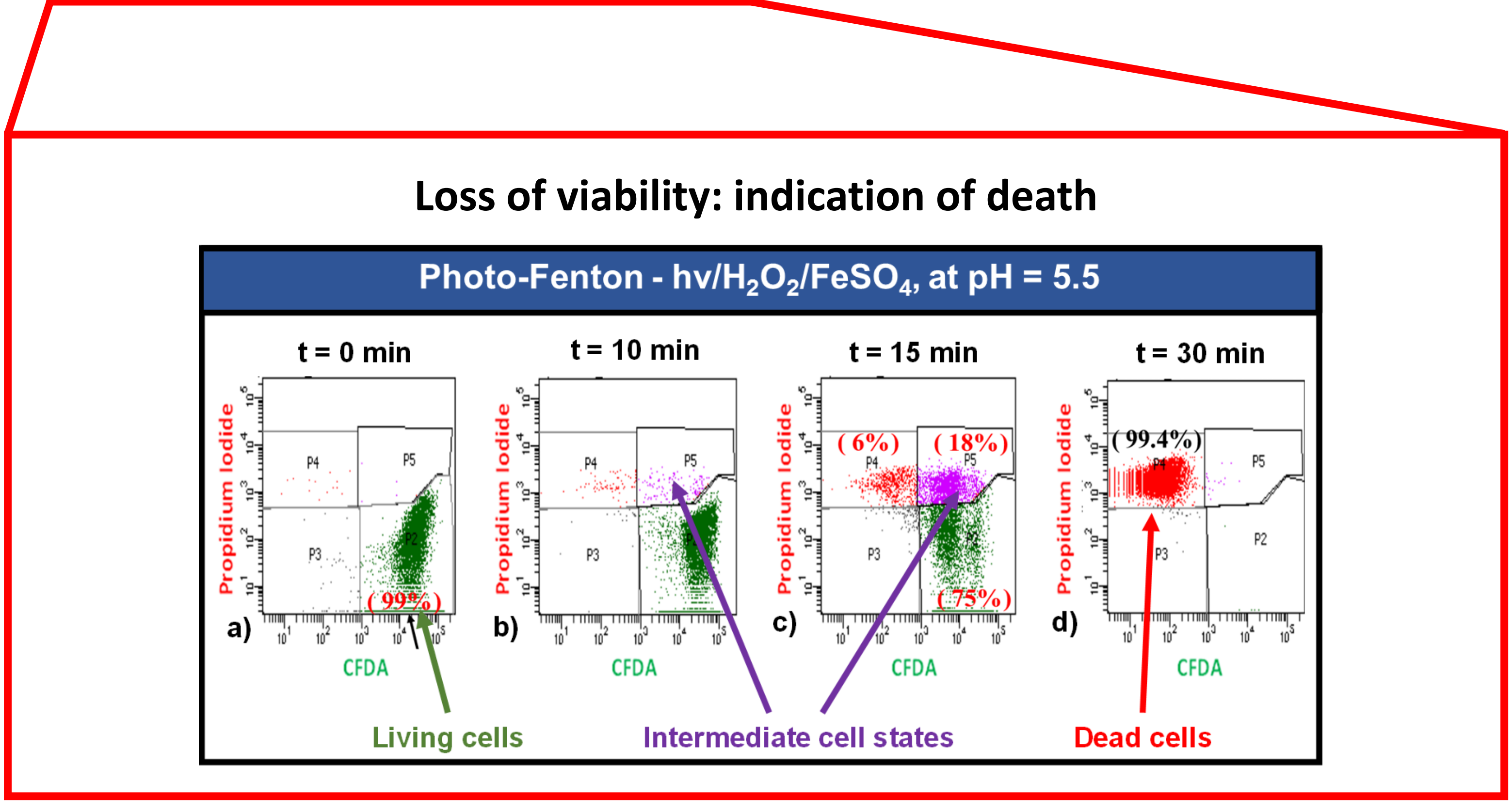
Establishing a timeline for $h\nu/H_2O_2/Fe$, verifies that…



Loss of viability: indication of death



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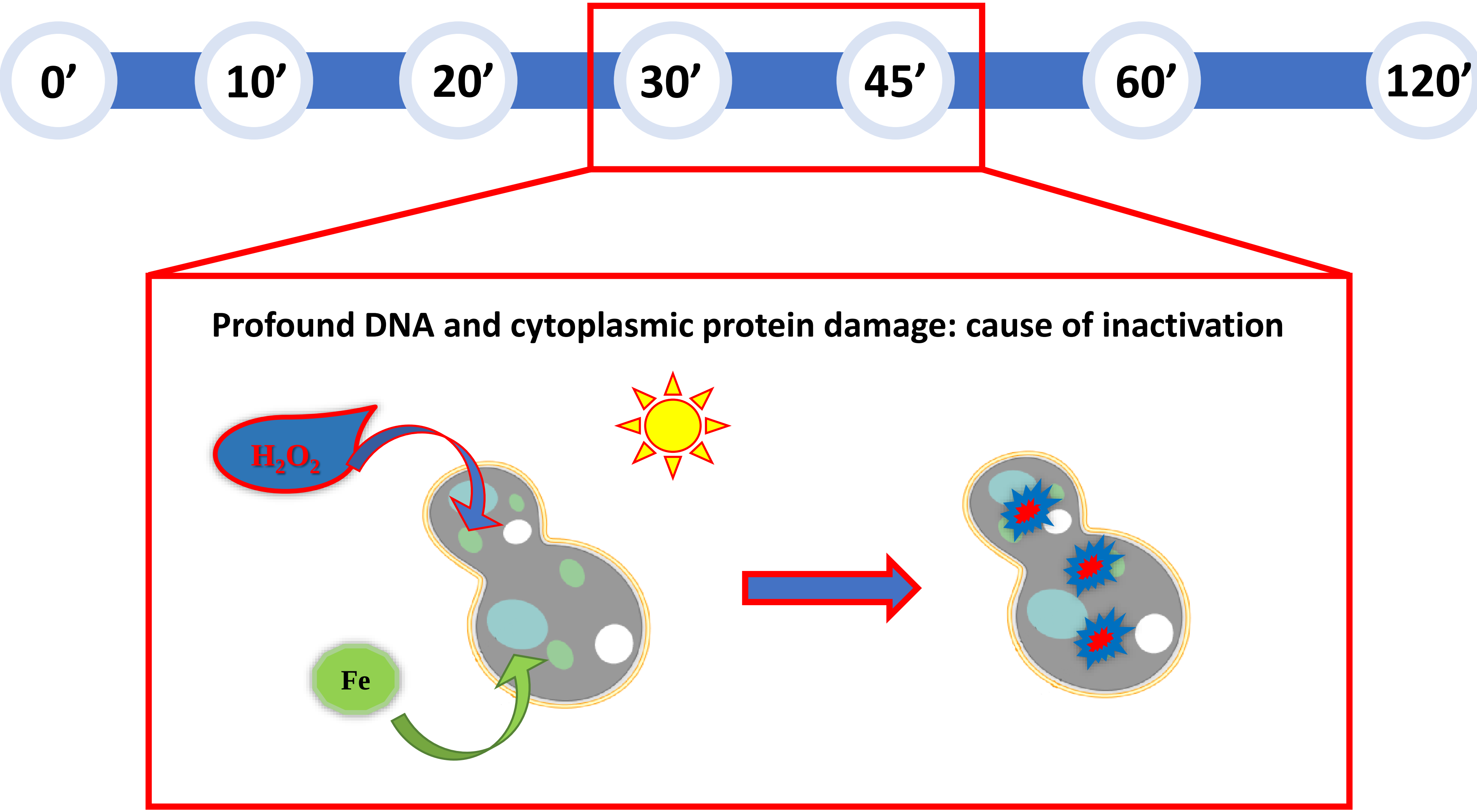
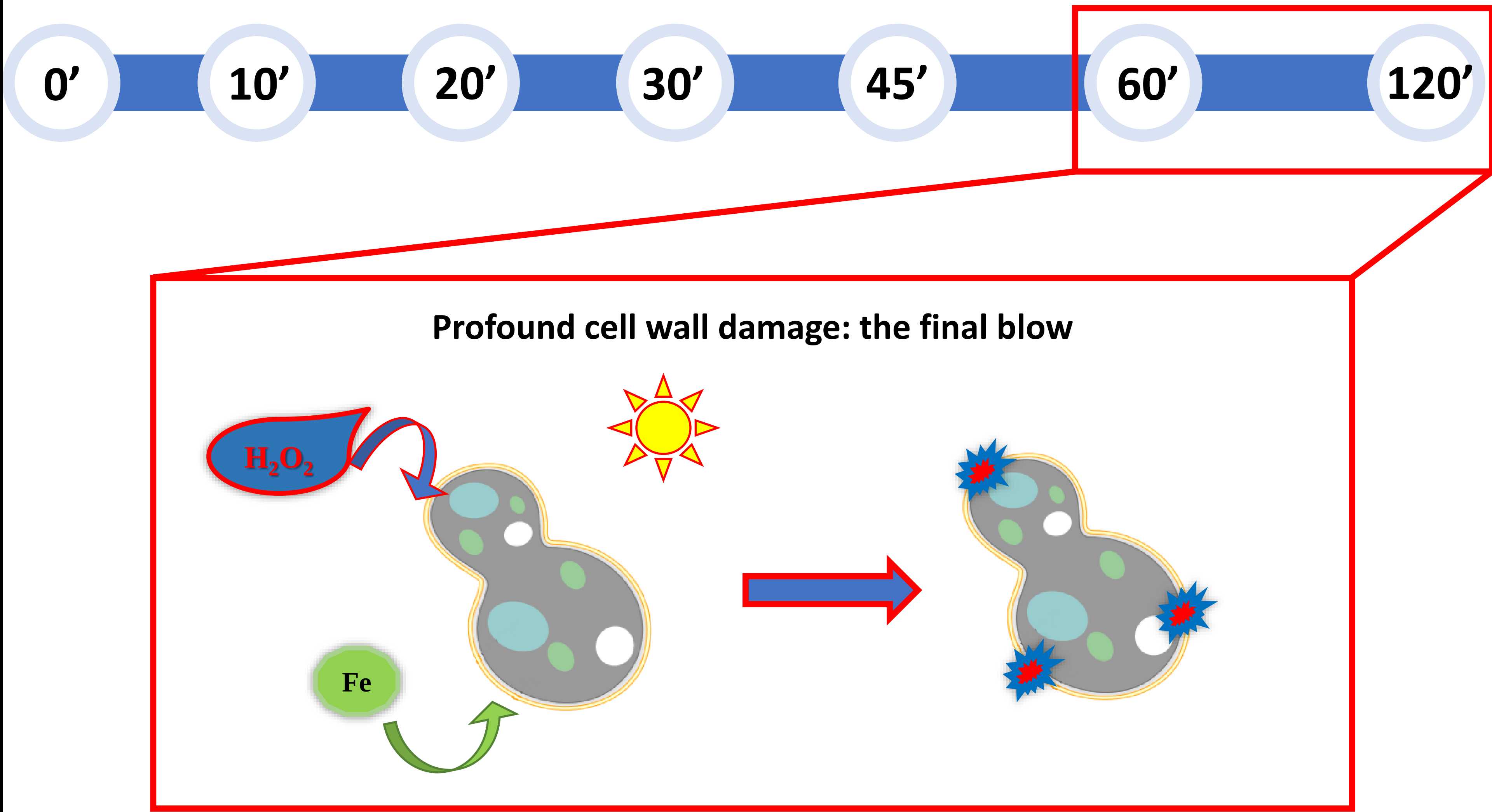
Establishing a timeline for hv/H_2O_2/Fe, verifies that…



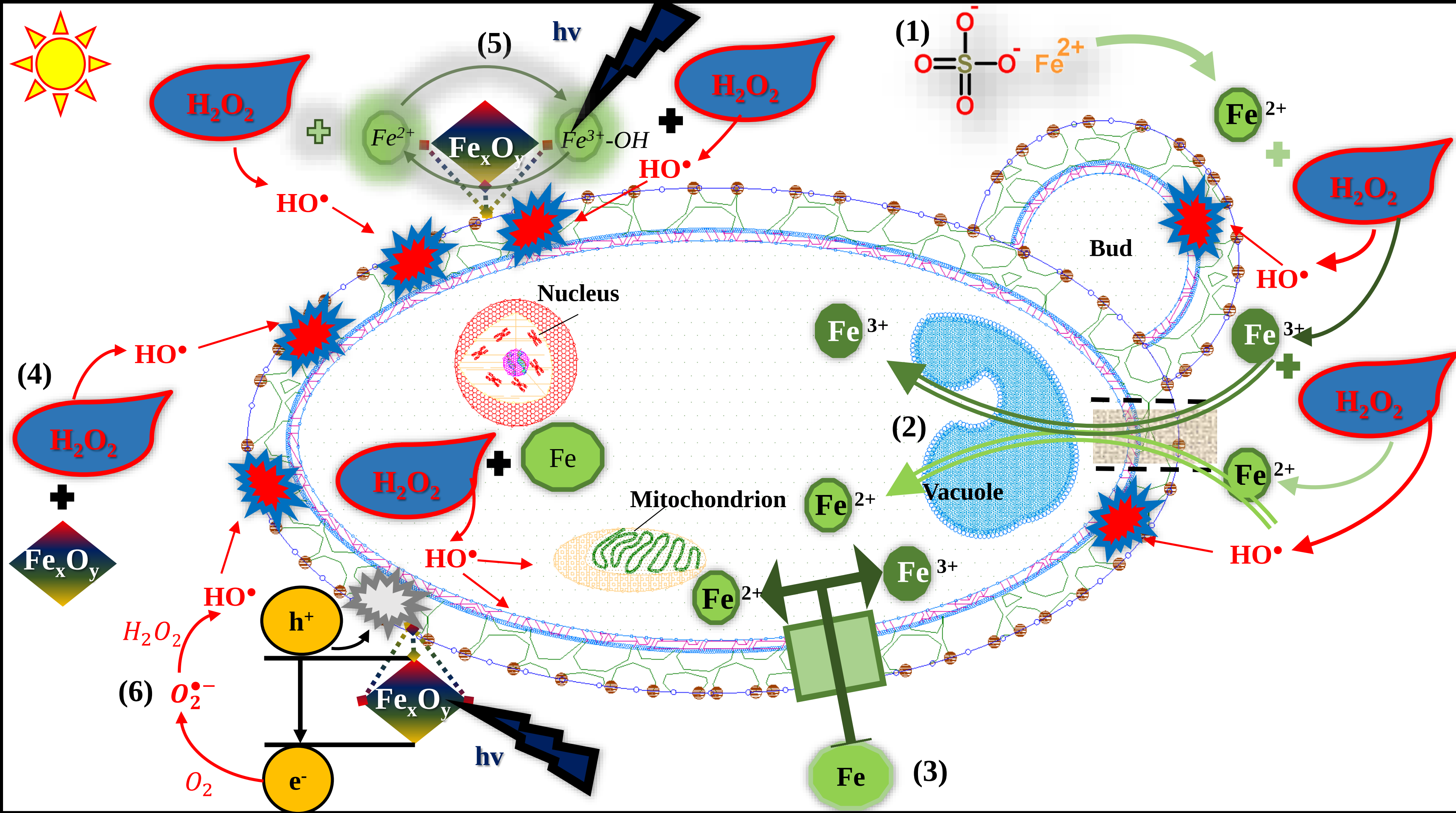
…internal photo-Fenton is the driving inactivation force!

Establishing a timeline for hν/H_2O_2/Fe, verifies that…



…internal photo-Fenton is the driving inactivation force!

Proposed inactivation mechanism

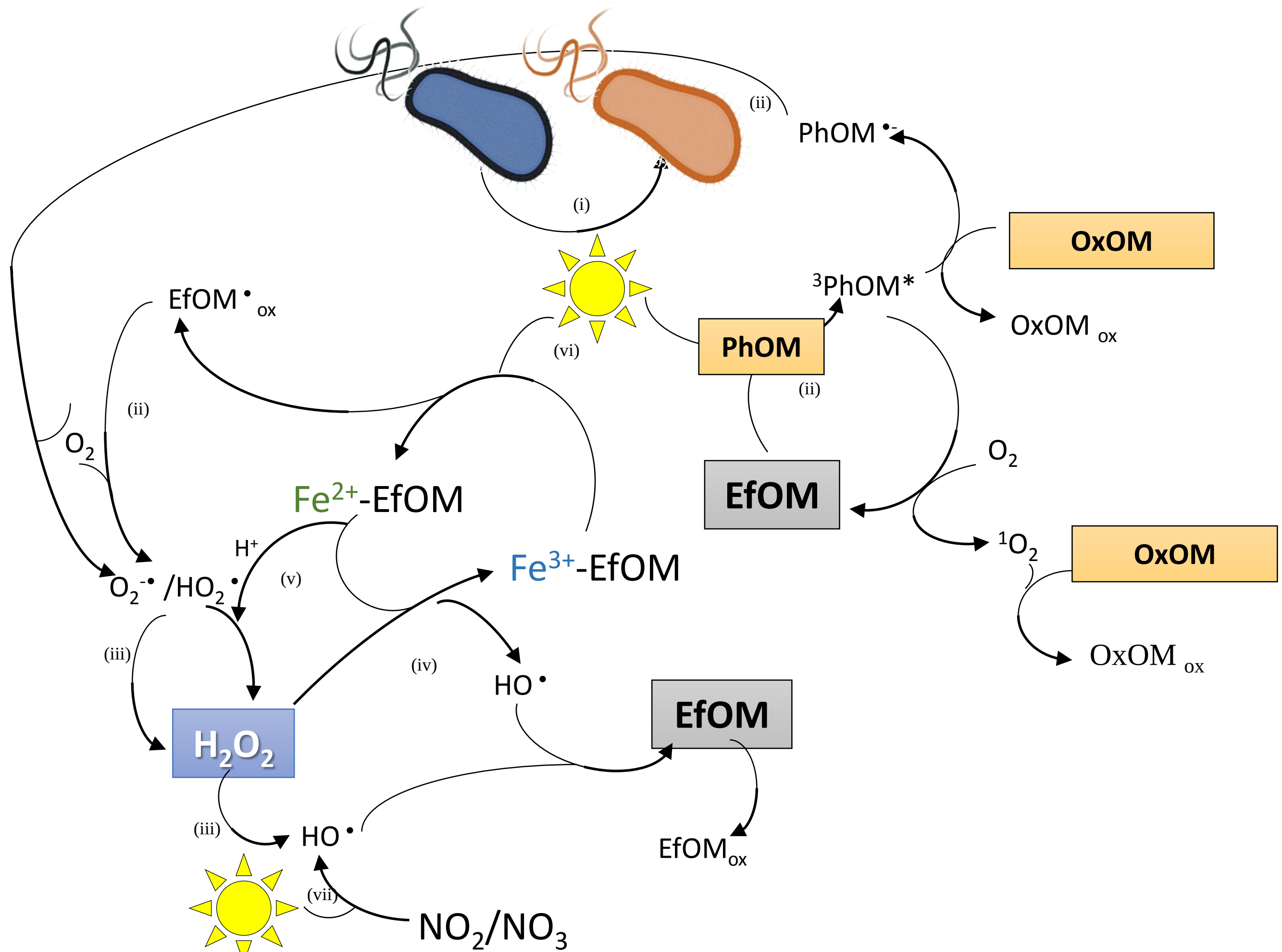


S. Giannakis, C. Ruales-Lonfat, S. Rtimi, S. Thabet, P. Cotton, C. Pulgarin, Applied Catalysis B: Environmental 185 (2016) 150-162.

Wastewater is…

- **Highly heterogeneous** — *Effluent Organic Matter (EfOM)*
- **Loaded with targets for light** — *Oxidizable Organic Matter (OxOM)*
- **Providing radical targets** — *OM and Microorganisms*
- **Containing photo-sensitizers** — *Photosensitizable Organic Matter (PhOM)*

Proposed degradation pathway



Abbreviations

EfOM:
Effluent Organic Matter

PhOM:
Photo-sensitizable fraction of EfOM

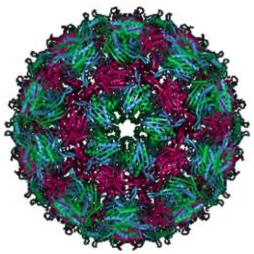
OxOM:
Oxidizable fraction of EfOM

(i)-(vii):
solar-induced pathways

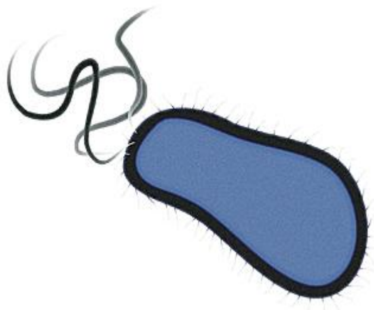
S. Giannakis, F.A. Gamarra Vives, D. Grandjean, A. Magnet, L.F. De Alencastro, C. Pulgarin, Water research 84 (2015) 295-306.

Summary: The time for >4-log inactivation

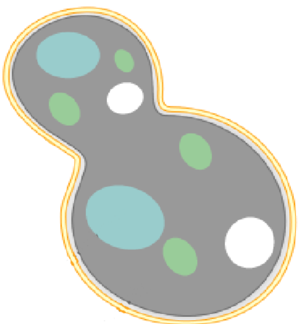
Male Somatic 2 coliphage



Escherichia coli **K-12**



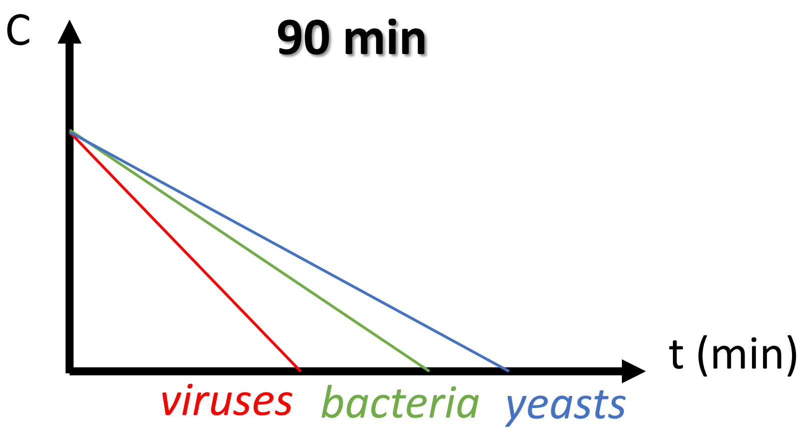
Saccharomyces Cerevisiae



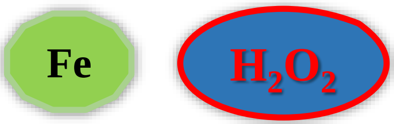
2 min

90 min

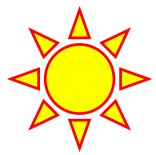
180 min



Attention: Dynamic response of the microorganisms

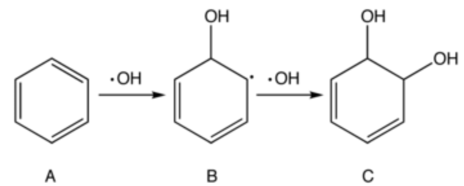


1^{st}, 2^{nd} order reaction rate

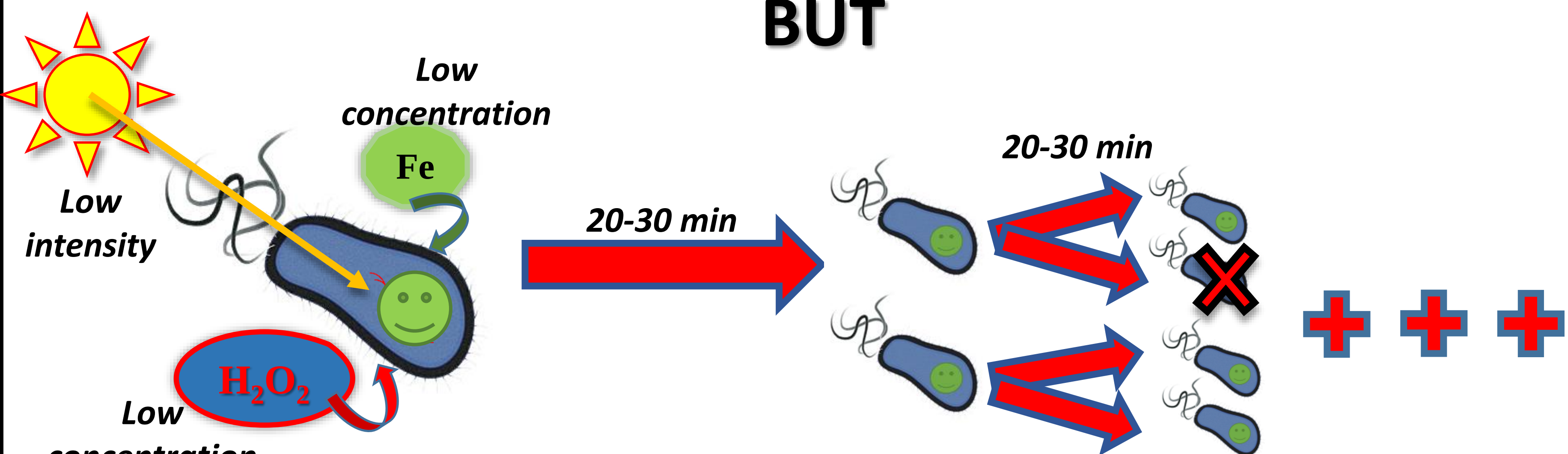


Reciprocity law, photolysis

Reaction constants



BUT



Take – home messages:

Mechanistic proposition photo-Fenton action mode

- *Cultivability*
- *Flow cytometry*
- *Use of single knock-out mutant strains*
- *DNA damages (Electrophoresis)*
- *Cell wall & internal protein degradation (Electrophoresis)*
- *Membrane peroxidation (MDA)*
- *Membrane integrity (ONPG)*
- *ROS generation (EPR, ESR)*
- *Literature*

Kinetics?

Thermodynamic aspects?

Proper controls?

View in the final application – Regrowth?

What do you want to prove?

Thank you for your attention, questions?

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