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

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Porto, 12 July 2017
Introduction? Lucky to present so late 😊

From Fenton...

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{HO}^\bullet + \text{OH}^- \]

\[ k_1=76 \text{ M}^{-1}\text{s}^{-1} \]

\[ \text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{HO}_2^\bullet + \text{H}^+ \]

\[ k_2=0.01 \text{ M}^{-1}\text{s}^{-1} \]

...to photo-Fenton

\[ \text{Fe}^{3+} + \text{H}_2\text{O} \xrightarrow{hv} \text{Fe}^{2+} + \text{H}^+ + \text{HO}^\bullet \]

Limiting step!
### Photo-catalytic significance of iron

<table>
<thead>
<tr>
<th>Reaction No.</th>
<th>Reaction</th>
<th>Reaction Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Fe$^{3+}$ + H$_2$O $\leftrightarrow$ Fe(OH)$^{2+}$ + H$^+$</td>
<td>$(k_1 = 2.9 \times 10^{-3} M)$</td>
</tr>
<tr>
<td>(2)</td>
<td>Fe$^{3+}$ + 2H$_2$O $\leftrightarrow$ Fe(ОН)$^2_2$ + 2H$^+$</td>
<td>$(k_2 = 7.62 \times 10^{-7} M^2)$</td>
</tr>
<tr>
<td>(3)</td>
<td>2Fe$^{3+}$ + 2H$_2$O $\leftrightarrow$ Fe$_2$(OH)$^{4+}$ + 2H$^+$</td>
<td>$(k_{22} = 0.8 \times 10^{-3} M)$</td>
</tr>
<tr>
<td>(4)</td>
<td>Fe$^{3+}$ + H$_2$O $\leftrightarrow$ Fe$^{3+}$(HO$_2$)$^{2+}$ + H$^+$</td>
<td>$(k_l = 3.1 \times 10^{-3})$</td>
</tr>
<tr>
<td>(5)</td>
<td>Fe(ОН)$^2_2$ + H$_2$O $\leftrightarrow$ Fe$^{3+}$(OH)(HO$_2$)$^+$ + H$^+$</td>
<td>$(k_l = 2 \times 10^{-4})$</td>
</tr>
<tr>
<td>(6a)</td>
<td>Fe$^{3+}$(HO$_2$)$^{2+}$ $\rightarrow$ Fe$^{2+}$ + HO$_2^*$</td>
<td>$(k_6 = x 10^{-3} s^{-1})$</td>
</tr>
<tr>
<td>(6b)</td>
<td>Fe$^{3+}$(OH)(HO$_2$)$^+$ $\rightarrow$ Fe$^{2+}$ + HO$_2^*$ + OH$^-$</td>
<td>$(k_6 = x 10^{-3} s^{-1})$</td>
</tr>
<tr>
<td>(7)</td>
<td>Fe$^{2+}$ + H$_2$O $\rightarrow$ Fe$^{3+}$ + HO$^*$ + OH$^-$</td>
<td>$(k_7 = 63 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(8)</td>
<td>Fe$^{2+}$ + HO$^*$ $\rightarrow$ Fe$^{3+}$ + OH$^-$</td>
<td>$(k_8 = 3.2 \times 10^8 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(9)</td>
<td>HO$^<em>$ + H$_2$O $\rightarrow$ HO$_2^</em>$ + H$_2$O</td>
<td>$(k_9 = 3.3 \times 10^9 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(10a)</td>
<td>Fe$^{2+}$ + HO$_2^*$ $\rightarrow$ Fe$^{3+}$(HO$_2$)$^{2+}$</td>
<td>$(k_{10a} = 1.2 \times 10^6 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(10b)</td>
<td>Fe$^{2+}$ + O$_2^*$ + H$^+$ $\rightarrow$ Fe$^{3+}$(HO$_2$)$^{2+}$</td>
<td>$(k_{10b} = 1 \times 10^7 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(11a)</td>
<td>Fe$^{3+}$ + HO$_2^*$ $\rightarrow$ Fe$^{2+}$ + O$_2$</td>
<td>$(k_{11a} &lt; 2 \times 10^3 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(11b)</td>
<td>Fe$^{3+}$ + O$_2^*$ $\rightarrow$ Fe$^{2+}$ + O$_2$</td>
<td>$(k_{11b} = 5 \times 10^7 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(12a)</td>
<td>HO$_2^<em>$ $\rightarrow$ O$_2^</em>$ + H$^+$</td>
<td>$(k_{12a} = 1.58 \times 10^5 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(12b)</td>
<td>O$_2^<em>$ + H$^+$ $\rightarrow$ HO$_2^</em>$</td>
<td>$(k_{12b} = 1 \times 10^{10} M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(13a)</td>
<td>HO$_2^<em>$ + HO$_2^</em>$ $\rightarrow$ H$_2$O$_2$ + O$_2$</td>
<td>$(k_{13a} = 8.3 \times 10^5 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(13b)</td>
<td>HO$_2^<em>$ + O$_2^</em>$ + H$_2$O $\rightarrow$ H$_2$O$_2$ + O$_2$ + OH$^-$</td>
<td>$(k_{13b} = 9.7 \times 10^7 M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(14a)</td>
<td>HO$^<em>$ + HO$_2^</em>$ $\rightarrow$ H$_2$O + O$_2$</td>
<td>$(k_{14a} = 0.71 \times 10^{10} M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(14b)</td>
<td>HO$^<em>$ + O$_2^</em>$ $\rightarrow$ O$_2$ + OH$^-$</td>
<td>$(k_{14b} = 1.01 \times 10^{10} M^{-1} s^{-1})$</td>
</tr>
<tr>
<td>(15)</td>
<td>HO$^<em>$ + HO$^</em>$ $\rightarrow$ H$_2$O$_2$</td>
<td>$(k_{15} = 5.2 \times 10^9 M^{-1} s^{-1})$</td>
</tr>
</tbody>
</table>

**Reactions initiated by iron**

*Initiation*

*Propagation*

*Termination*

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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
Our models...

Male Somatic 2 Coliphage

Escherichia coli K-12

Saccharomyces Cerevisiae

\[ \times 50 \text{ times} \]

\[ \times 5 \text{ times} \]

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

27.5 nm

1 – 2 μm

5-10 μm
1) Action of solar light: baseline, and an AOP in disguise
2) Addition of $\text{H}_2\text{O}_2$
3) Addition of Iron $\text{Fe}^{2+}, \text{Fe}^{3+}, \text{Fe}_x\text{O}_y$
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 is an “indirect” AOP
ONLY BY SOLAR LIGHT

E. coli

ROS (HO•, O2, H2O2) + Fe2+ → HO• + Fe3+

External
PS \to \text{PS}^*

Internal
PS \to \text{PS}^*

ROS attacks

\text{Excited state} \to \text{ROS attacks}

\text{O}_2

\text{Fe}^{3+}\to \text{Fe}^{2+}

\text{hv}

hv

\text{photo-Fenton reaction}

\text{DNA}

\text{UVB Direct action}

\text{UVA}

\text{ONLY BY SOLAR LIGHT}
Solar light + H$_2$O$_2$
Solar light + $\text{H}_2\text{O}_2$ + Fe
Previous work on bacteria

The effect of Fe$^{2+}$, Fe$^{3+}$, H$_2$O$_2$ 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 Pulgarín.
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...


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.

Iron oxides semiconductors are efficiencies 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

Results:
I: TiO$_2$ photocatalysis
II: pF with FeSO$_4$
III: hv/FeSO$_4$
IV: hv/H$_2$O$_2$
V: Solar light
VI: Fenton (dark)

Cultivability

MDA formation

Cell wall degradation

Internal damage

Integrated mechanism

Previous work on viruses

Principal parameters affecting virus inactivation by the solar photo-Fenton process at neutral pH and μM concentrations of H₂O₂ and Fe²⁺/³⁺


Fe/H₂O₂ ratio: 1/1 I: 300 W/m²
Proposed inactivation mechanism

Key to inactivation: iron complexation with HA

Available iron throughout the test!

Photo-Fenton takes place!

Yeast Inactivation: a brief summary in MQ

- \( \text{hv/H}_2\text{O}_2/\text{FeSO}_4 \): mainly homogeneous external process
- \( \text{hv/H}_2\text{O}_2/\text{Fe-Oxides} \): goethite-natural semiconductor or heterogeneous mode of action
- \( \text{hv/H}_2\text{O}_2/\text{Fe-Citrate} \): mild complex achieving homogeneous process in higher pH

DNA and protein damages (photo-Fenton process)

$hv/FeSO_4/H_2O_2$ at $pH = 5.5$

Cell wall proteins

<table>
<thead>
<tr>
<th>0’</th>
<th>30’</th>
<th>60’</th>
<th>120’</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>130</td>
<td>95</td>
<td>72</td>
</tr>
<tr>
<td>72</td>
<td>55</td>
<td>43</td>
<td>34</td>
</tr>
<tr>
<td>34</td>
<td>26</td>
<td>17</td>
<td>10</td>
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</table>

DNA

<table>
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<tr>
<th>0’</th>
<th>30’</th>
<th>45’</th>
<th>60’</th>
<th>120’</th>
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</thead>
<tbody>
<tr>
<td>DNA band pattern for each time point</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Cytoplasmic proteins

<table>
<thead>
<tr>
<th>0’</th>
<th>30’</th>
<th>45’</th>
<th>60’</th>
<th>120’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein band pattern for each time point</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Establishing a timeline for hv/H$_2$O$_2$/Fe, verifies that...

...internal photo-Fenton is the driving inactivation force!
Establishing a timeline for hv/H₂O₂/Fe, verifies that...

Loss of cultivability: 1st indication of inactivation

Budding stops

...internal photo-Fenton is the driving inactivation force!
Establishing a timeline for hv/H_2O_2/Fe, verifies that...

Loss of viability: indication of death

...internal photo-Fenton is the driving inactivation force!
Establishing a timeline for hv/H$_2$O$_2$/Fe, verifies that...

0'  10'  20'  30'  45'  60'  120'

Profound DNA and cytoplasmic protein damage: cause of inactivation

...internal photo-Fenton is the driving inactivation force!
Establishing a timeline for hv/H$_2$O$_2$/Fe, verifies that...

...internal photo-Fenton is the driving inactivation force!
Proposed inactivation mechanism

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

Summary: The time for >4-log inactivation

- Male Somatic 2 coliphage: 2 min
- *Escherichia coli* K-12: 90 min
- *Saccharomyces Cerevisiae*: 180 min

The graph shows the inactivation of viruses, bacteria, and yeasts over time.
Attention: Dynamic response of the microorganisms

Fe \quad \text{H}_2\text{O}_2

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

Reciprocity law, photolysis

BUT

Low intensity
Low concentration

20-30 min
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?

More info:
Dr. Stefanos Giannakis, E-mail: stefanos.giannakis@epfl.ch

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement number 688928.