TRANSFER OF PHOTOOXIDATIVE PROCESSES FROM SENESCENT PHYTOPLANKTON CELLS TO ATTACHED BACTERIA : IMPLICATIONS ON THE PRESERVATION OF ORGANIC MATTER

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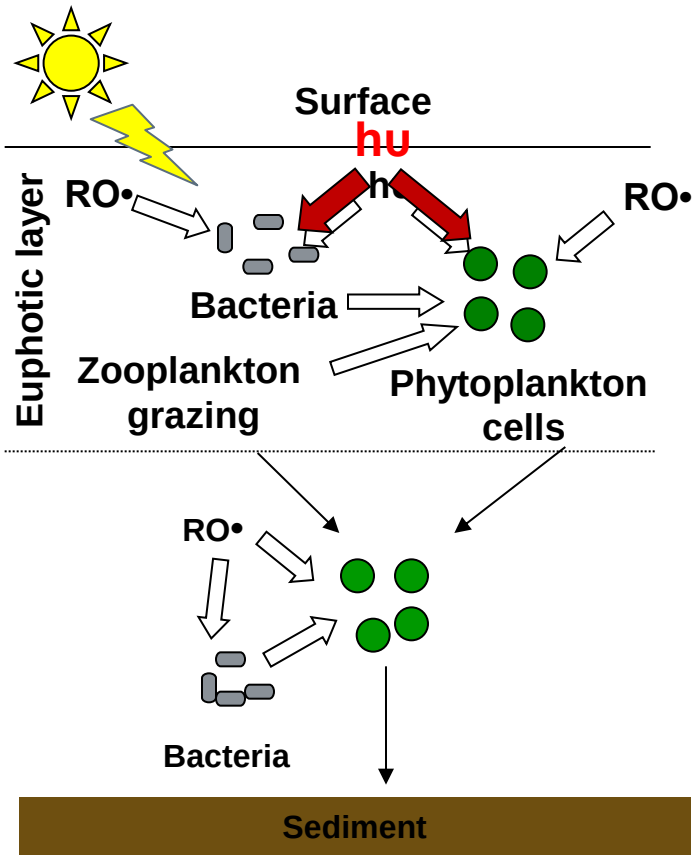
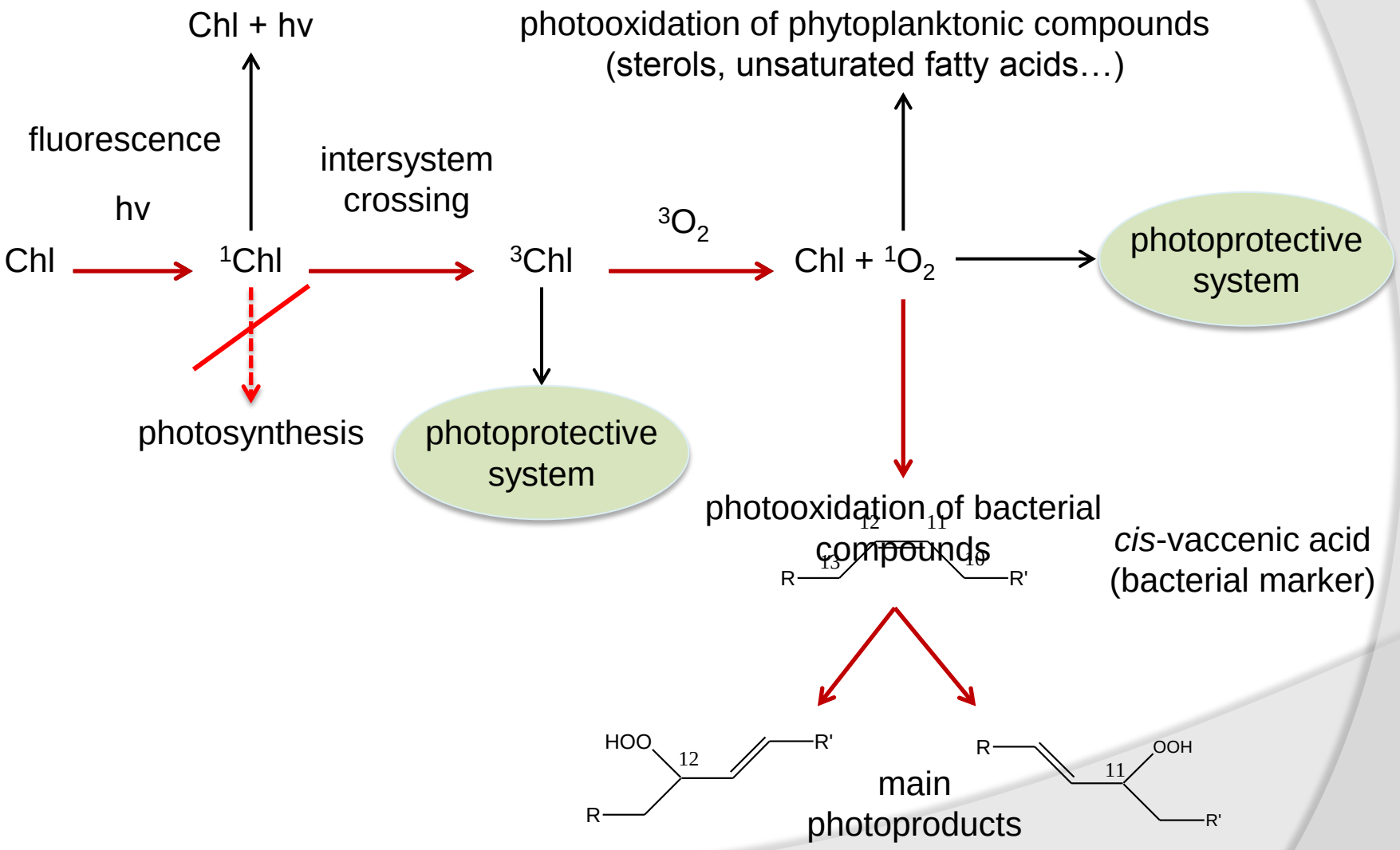
Biotic and abiotic process in ocean



- bacteria and phytoplankton cells in the euphotic layer are influenced by different biological and photochemical processes

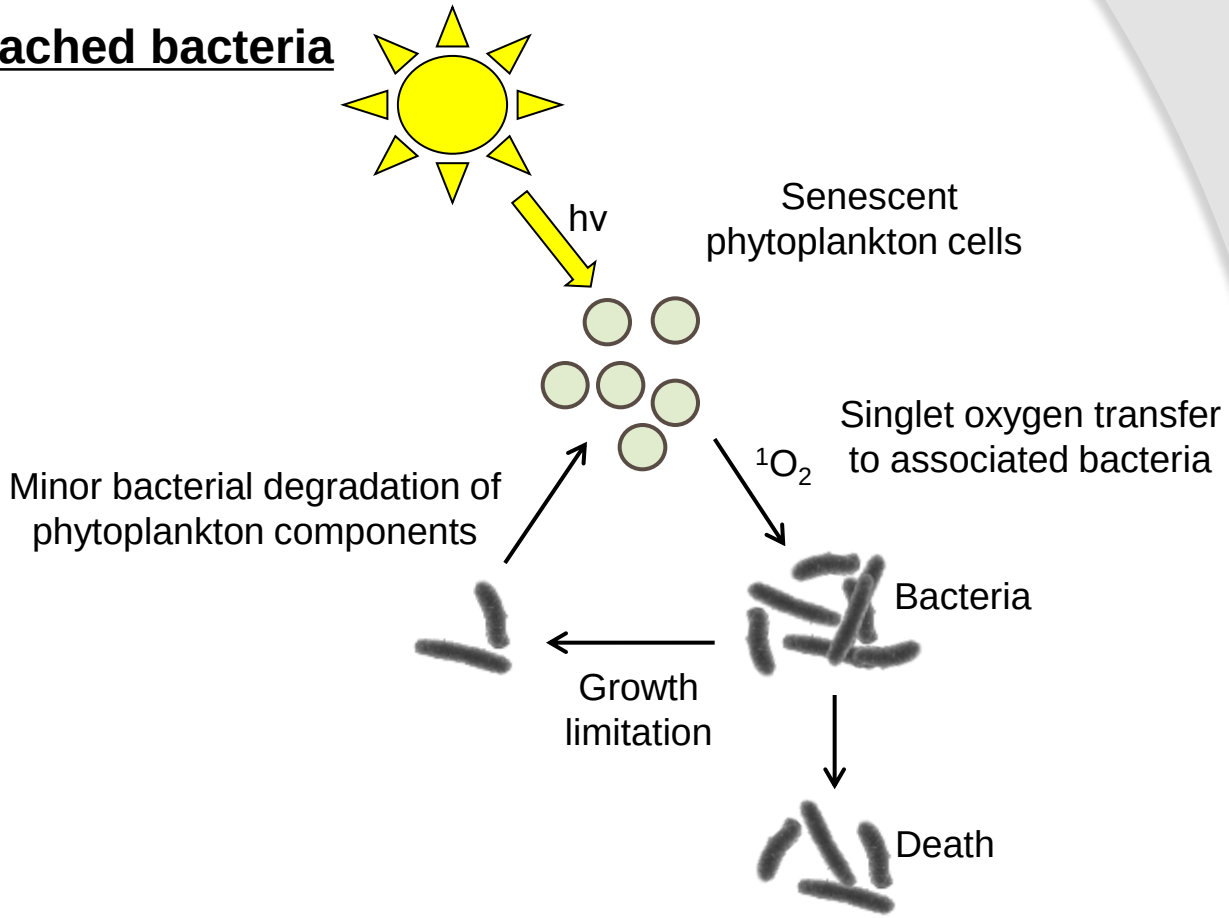
- photochemistry processes important way of degradation : until 40% of the O_2 produced during photosynthesis could be used for photooxidation (Laane & al, 1985)

On the origin of singlet oxygen $^1O_2$



Rontani, 2001

Transfer of $^1O_2$ to attached bacteria



Hypothesis :

- Transfer of singlet oxygen to attached bacteria during the senescence of phytoplankton cells
- Limitation of bacterial growth
- Minor degradation of phytoplankton components

Photodegradation of bacteria associated with *Emiliania huxleyi* (*Eh*)

Experiment :

- Non axenic culture of *Eh* was incubated under controlled conditions until stationary phase. Cell senescence induced by transferring cells in old seawater (after centrifugation)

- Samples incubated for 2 days in solar simulator (Atlas Suntest simulator, 450W.m^{-2} equivalent of sun radiation in Mediterranean Sea in summer)

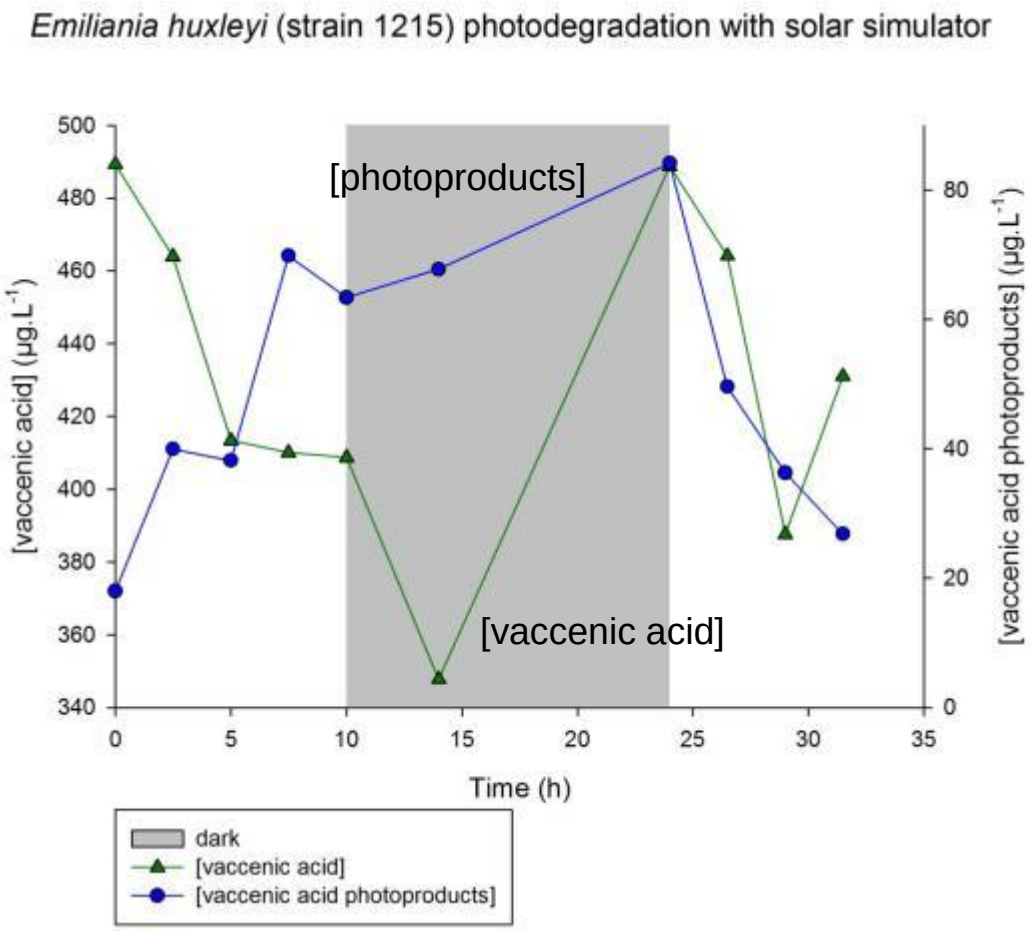
Analysis :

- *Cis*-vaccenic acid and photoproducts analyzed by GC/MS (Agilent 5975C mass spectrometer connected to a 6850 gas chromatograph)

- Bacterial and phytoplankton abundance measured in Flow Cytometer (PRECYM : http://precym.com.univ-mrs.fr/)

- Chlorophyll *a* measured by fluorimetry

Results :



Light period (0 - 10h) :

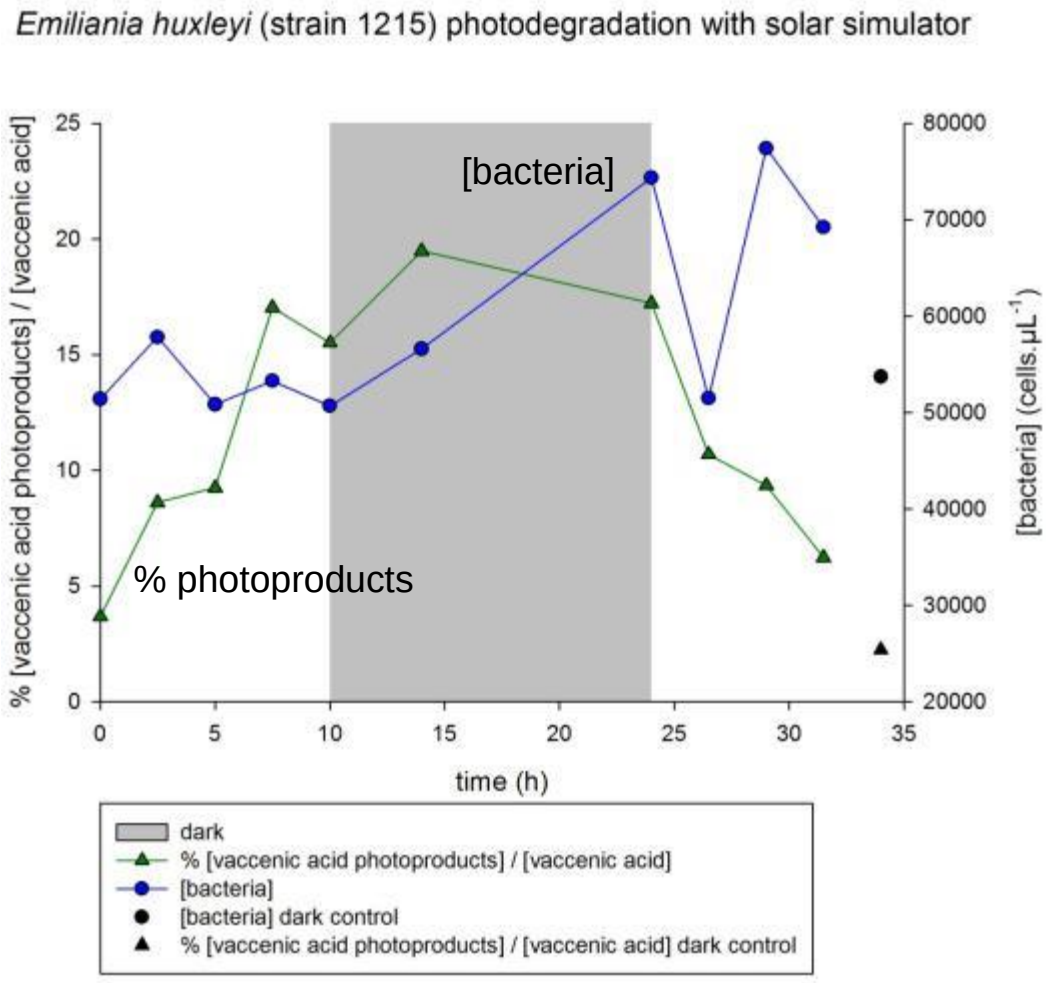
- decrease of cis-vaccenic acid correlated with an increase of photoproducts

Dark period (10 - 24h) :

- photoproduct formation slows down
- bacterial community increases as shown by *cis*-vaccenic increase

- Transfer of singlet oxygen from phytodetritus to bacteria
- This transfer happens during exposure to light

Results :



Light period (0 - 10h) :

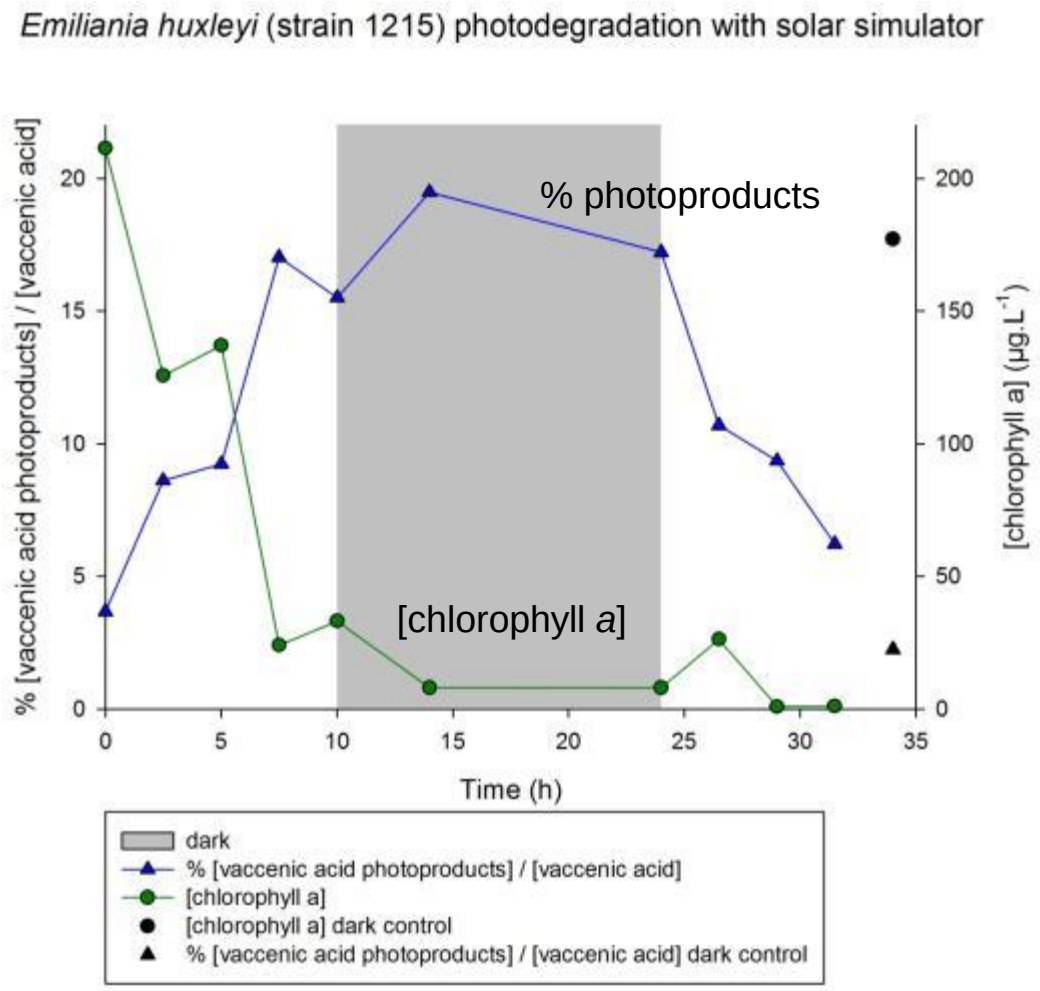
- [bacteria] constant
- increase of % [vaccenic acid photoproducts]

Dark period (10 - 24h) :

- % [vaccenic acid photoproducts] reaches a constant value and [bacteria] increases from 50,000 to 75,000 cells.µL^{-1}

Singlet oxygen transfer seems to stop bacterial growth during exposure to light

Results :



Light period (0 - 10h) :

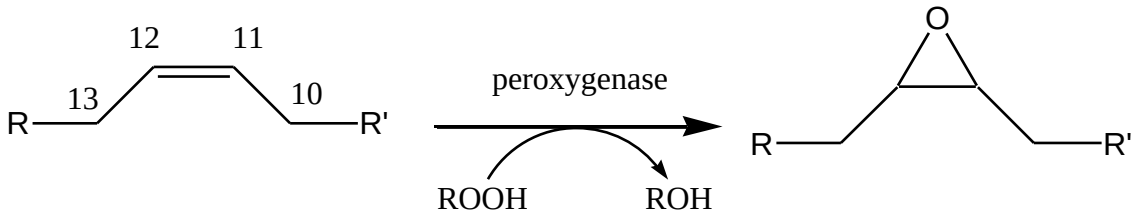
- Decrease of [chlorophyll *a*] from 200 to 25 µg.L^{-1}
- Increase of % *cis*-vaccenic acid photoproducts from 4 to 16%

Dark period (10 - 24h) :

- % *cis*-vaccenic acid photoproducts constant around 18%
- [chlorophyll *a*] is around 8 µg.L^{-1} after 15h.

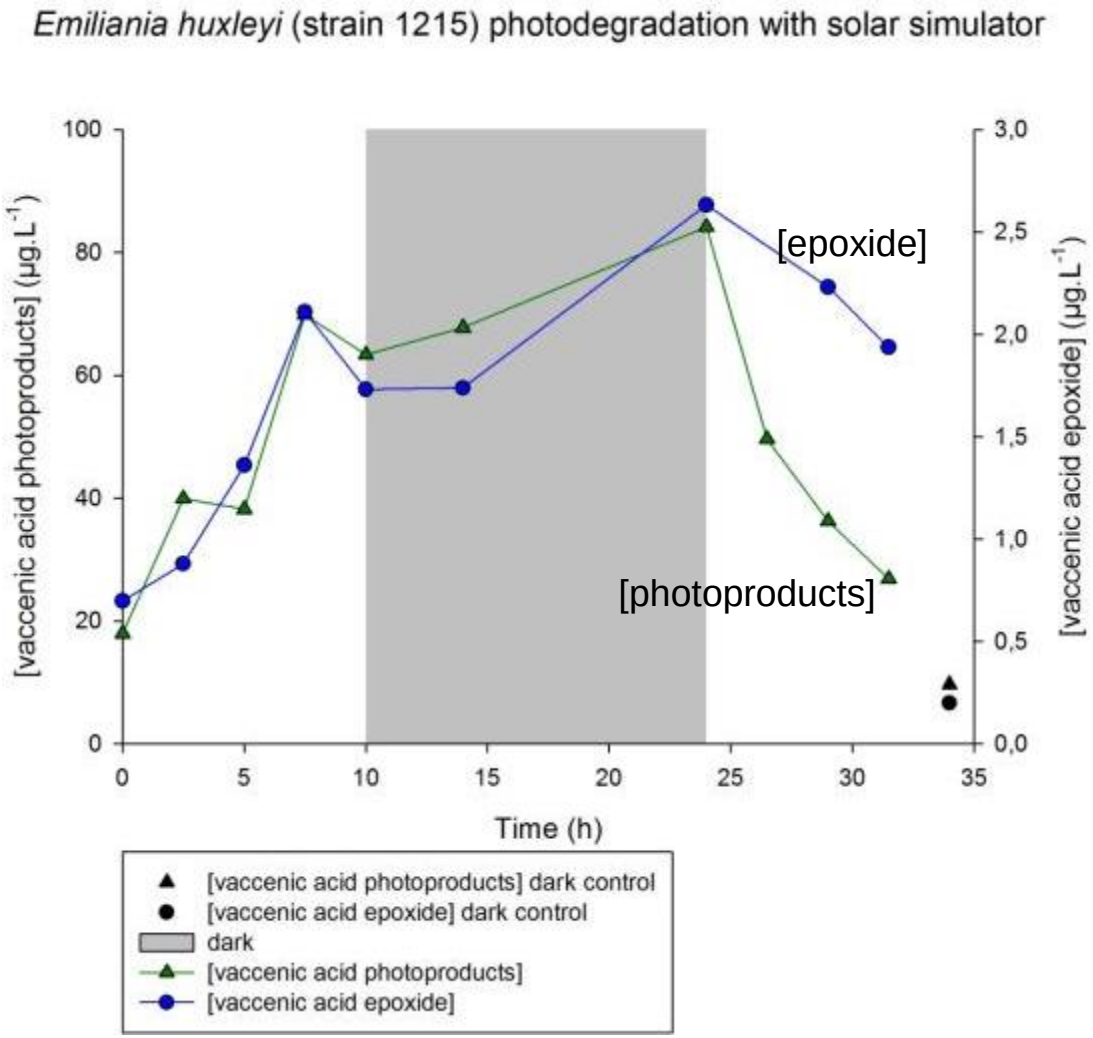
- Photooxidation of *cis*-vaccenic acid linked to photooxidation of chlorophyll *a*
- Confirmation of singlet oxygen transfer

- Production of vaccenic acid epoxide during the experiment.
- Formation of this compound by addition of a peroxyl radical to the double bond is very unlikely
- Could be explained by peroxygenase activity of bacteria (Piazza, 1999; Blée, 1990)



- Process could be used by some bacteria to minimize the hydroperoxide toxicity

Results :

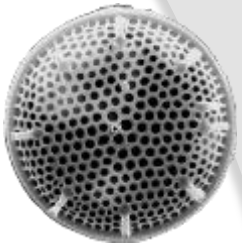


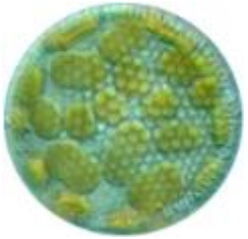
- Considerable increase of [vaccenic acid photoproducts] and [vaccenic acid epoxide] during the experiment

Bacterial response (peroxygenase) to minimize damage induced by photochemically produced hydroperoxides

Diatoms specific characters :

- Major group of phytoplankton
- Cell which built a rigid cell wall made of amorphous silica (frustule)
- Frustule acts as a polar matrix (charged surface)





Hypothesis :

Charged mineral surfaces, such as siliceous diatom frustules, may reduce the lifetime of $^1O_2$ and allow for enhanced bacterial growth and biodegradation

Experiments :

Photodegradation of bacteria associated with *Emiliania huxleyi* (*Eh*) cells

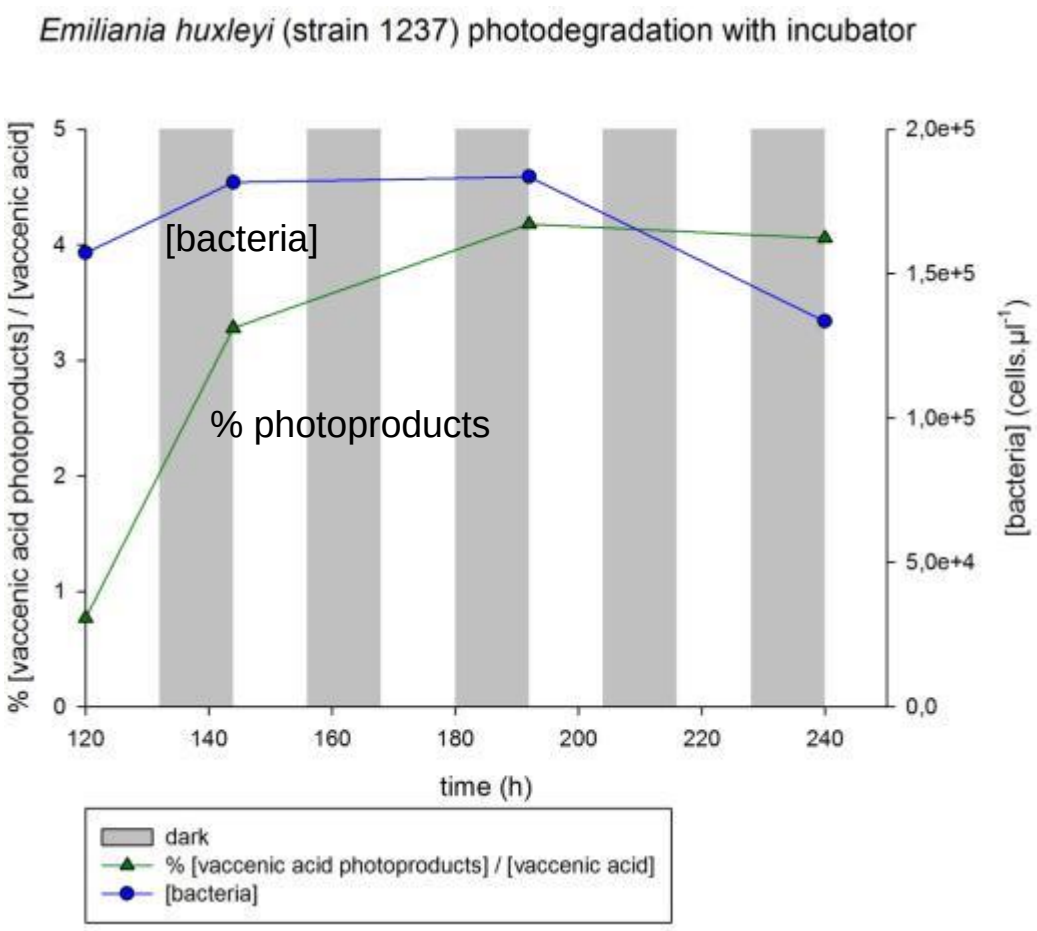
- Non-axenic culture of *Eh* incubated in controlled conditions (light/dark 12/12, 17° C) until stationary phase
- Samples incubated under light/dark cycle (12h/12h, 36 $W.m^{-2}$) for 5 days

Photodegradation of bacteria associated with *Haslea ostrearia* (*Ho*) cells

(provided by Dr. S. Belt – Plymouth University)

- Lyophilized culture of *Ho* + marine bacterial inoculum in f/2 medium
- Samples incubated under light/dark cycle (12h/12h, 36 $W.m^{-2}$) for 5 days

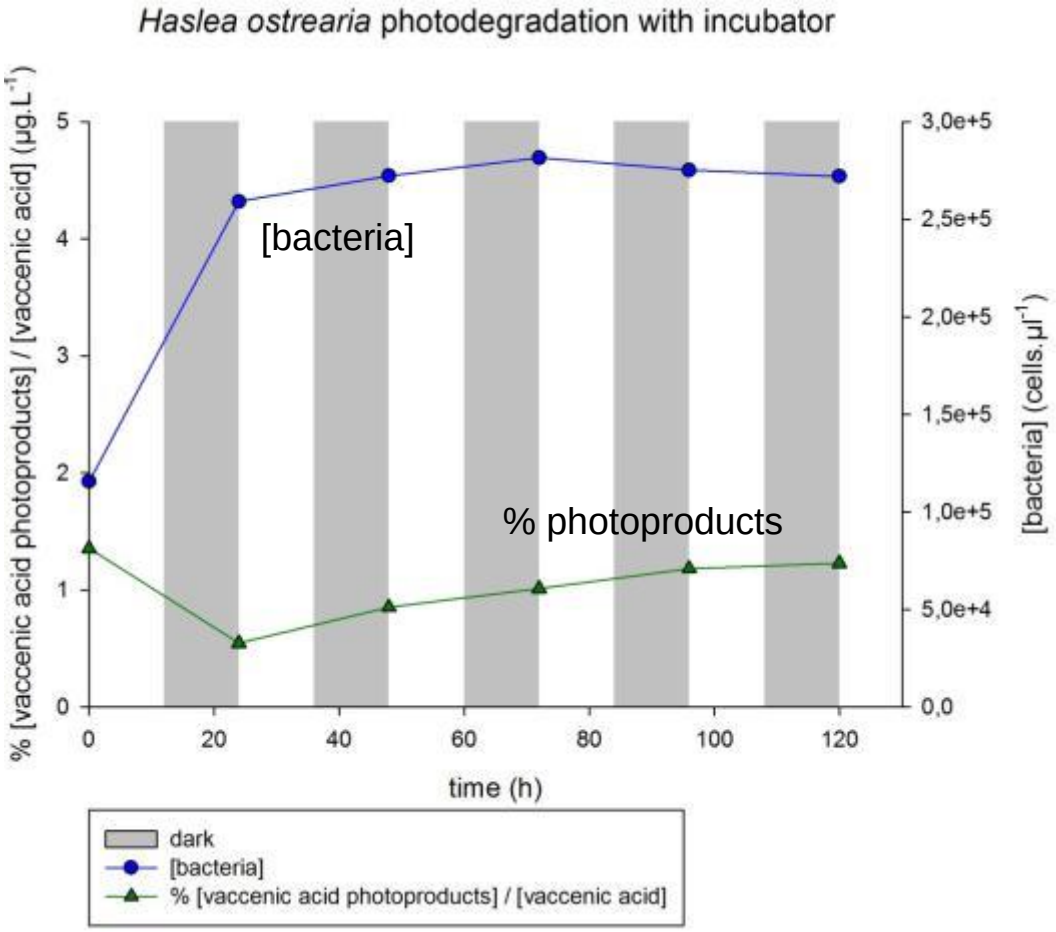
Results :



- bacterial concentration stays constant while there is an initial rapid increase in % *cis*-vaccenic acid photoproducts

The singlet oxygen transfer from phytodetritus to attached bacteria is effective

Results :



- large increase of bacterial abundance during the first 24h
- low % of vaccenic acid photoproducts which stays approximately constant

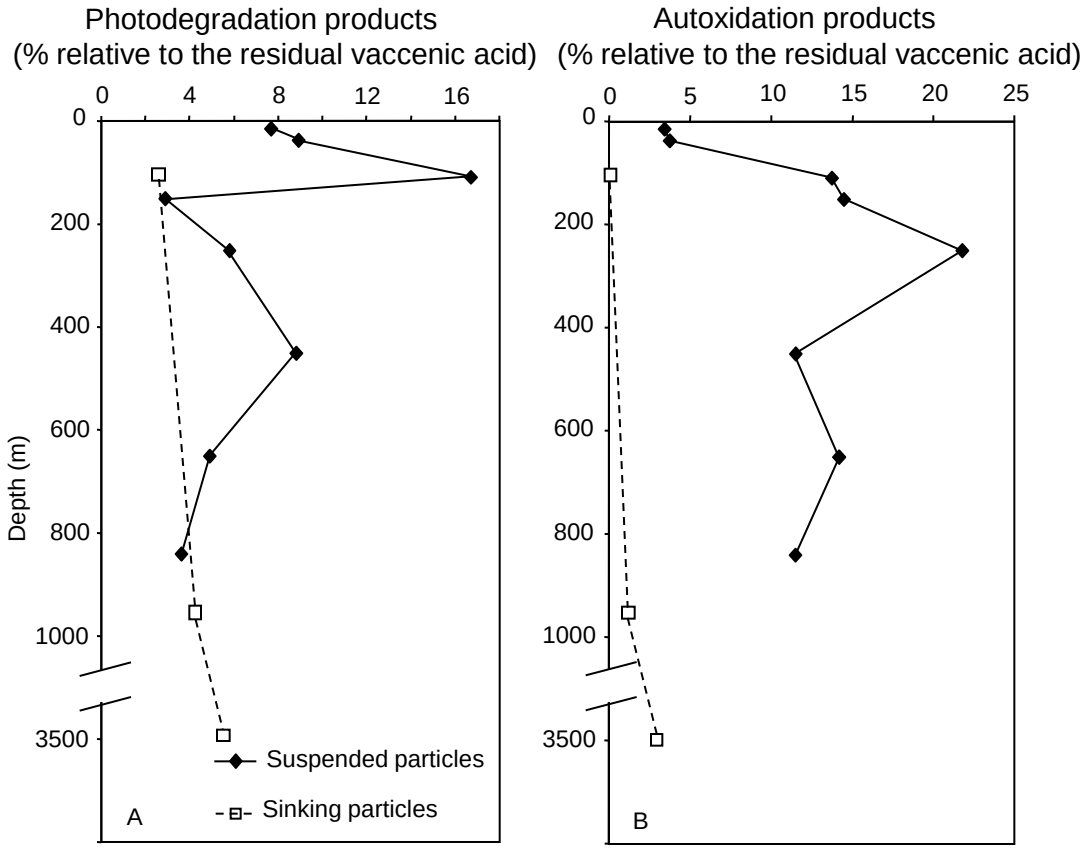
Polar matrices like diatoms frustules could stop or slow down the singlet oxygen transfer from phytodetritus to attached bacteria

Hypothesis :

Singlet oxygen ($^1O_2$) transfer from phytodetritus to attached bacteria could be responsible for the better preservation of POM previously observed in suspended particles in Equatorial Pacific (U.S. Joint Global Ocean Flux Study; JGOFS 1992) (Wakeham *et al*., 1997).

Approach :

- Reinvestigation of the behavior of organic matter (OM) in suspended and sinking particles in the equatorial Pacific Ocean (0° N, 140° W) by measuring lipid degradation products that are specific for biodegradation and photoxidation.
- Suspended particles : *in situ* large volume filtration system at the equator site 8 depths (15-850 m).
- Sinking particles : sediment trap samples collected at 105, 955 and 3459 m

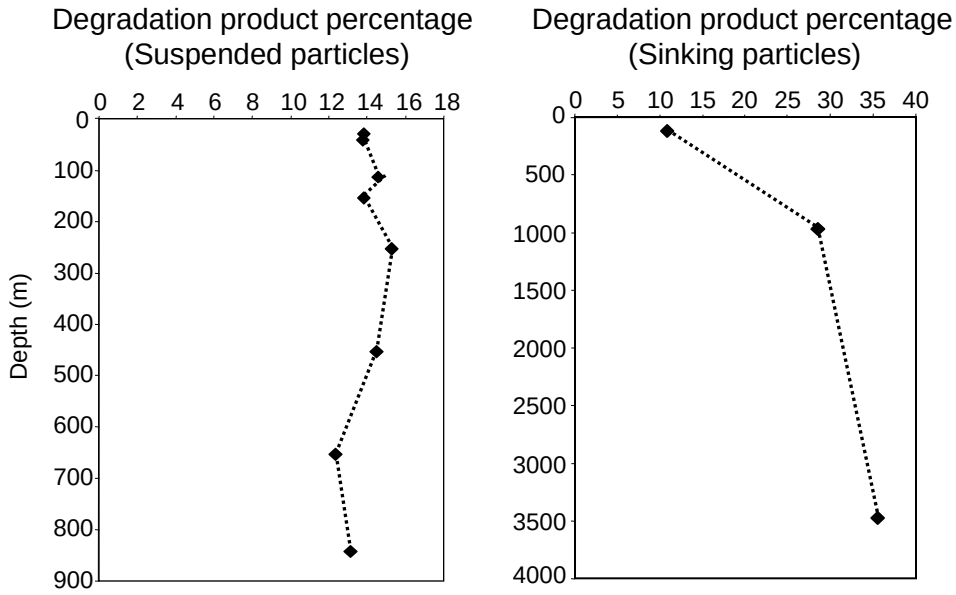


- very weak photooxidation and autoxidation of *cis*-vaccenic acid in sinking particles containing high proportion of diatoms.
- *cis*-vaccenic acid was significantly photodegraded in the suspended particles exiting the photic zone (collected at 110 m) (Fig. A)
- strong autoxidation observed in suspended particles collected below the photic zone between 110 and 250 m (Fig. B).

Transfer of $^1O_2$ from phytodetritus to attached bacteria is apparently more efficient for suspended particles than in diatom-rich sinking particles

Rontani *et al.*, 2011

Monitoring of the biotic degradation of 24-Methylcholesta-5,22-dien-3*b*-ol degradation product percentage (specific marker of phytoplankton) (Volkman, 1986 ; 2003)



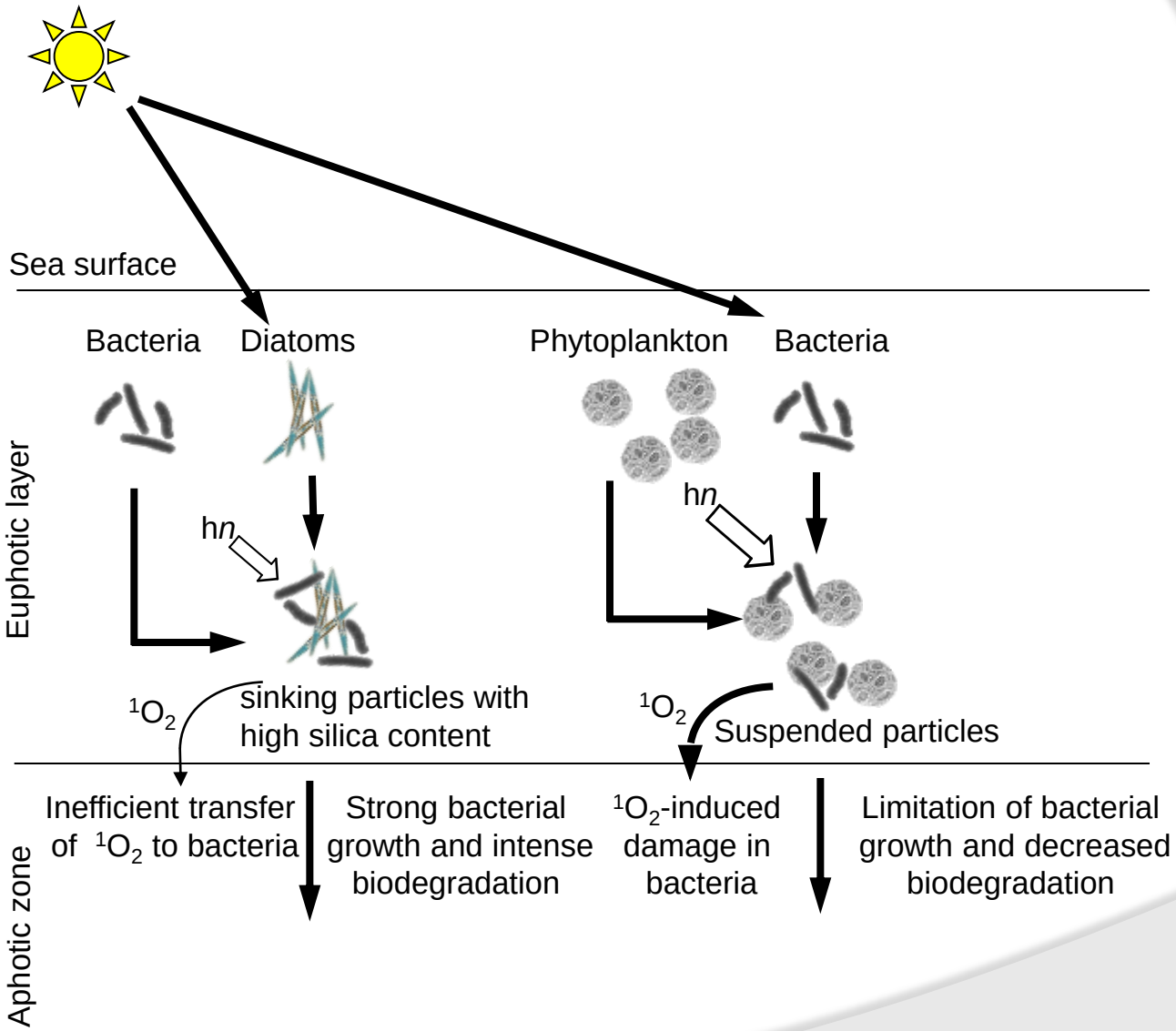
Biodegradation product % (% of stanol and Δ^4-sterone relative to the parent sterol)

- biotic degradation relatively low (~14%) and constant with depth in suspended particles
- strong increase of biotic degradation (up to 35%) in sinking particles

Phytodetritus may be better preserved against biodegradation in suspended particles than in fast-sinking particles as a consequence of bacterial oxidation state

Rontani *et al.*, 2011

Conclusion



Adapted from Rontani *et al*., 2011

THANK YOU FOR YOUR ATTENTION