Observation and manipulation of biological objects on plastic films immersed in sea water

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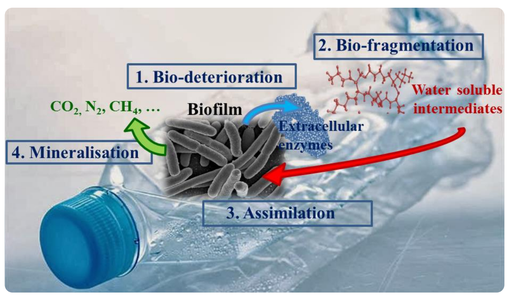
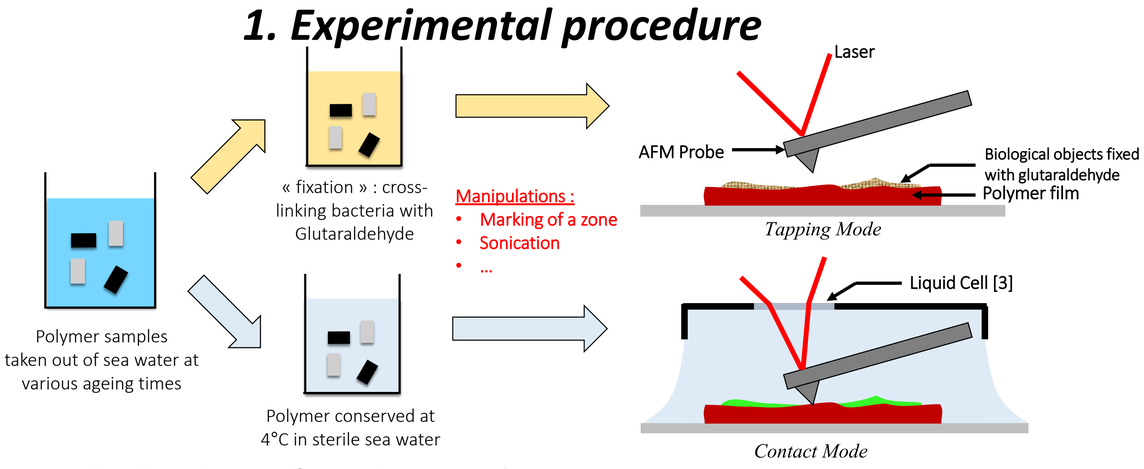


The different steps of plastic biodegradation by microorganisms [1]

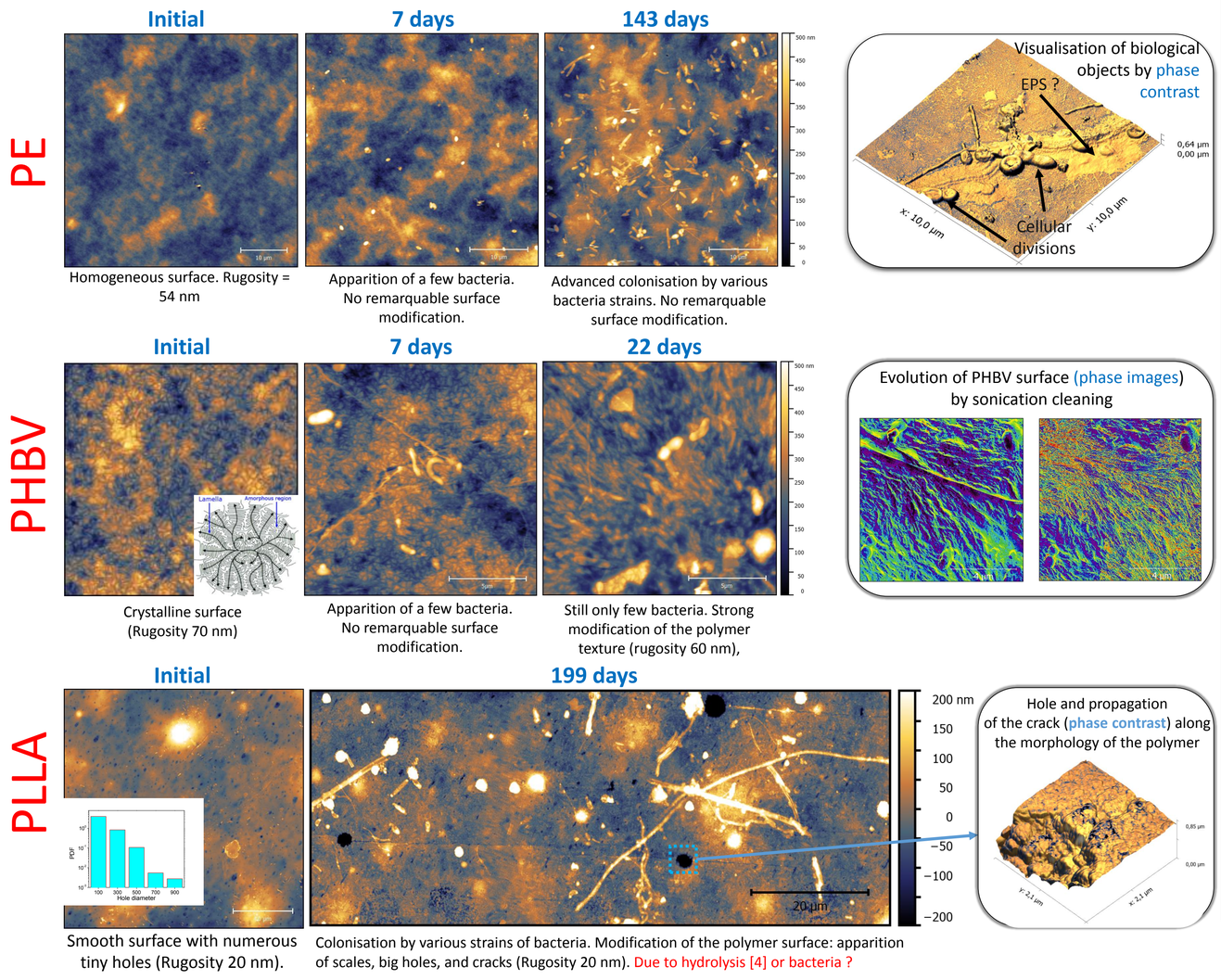
Pollution by plastic litter has become a major environmental problem in recent years, as they accumulate in a dramatic way in terrestrial and marine environments [1]. **Biodegradable** polymers is one of the major direction taken to produce more "eco-friendly" materials, yet the real bio-degradability of these polymers remains to be proven. Among all ageing processes [2] we focus here on the role of **biological objects** on the fate of several polymer in various conditions, using AFM imaging technique to follow the **apparition of micron-sized defects**, such as cracks and holes.

Polymer ageing in sea water

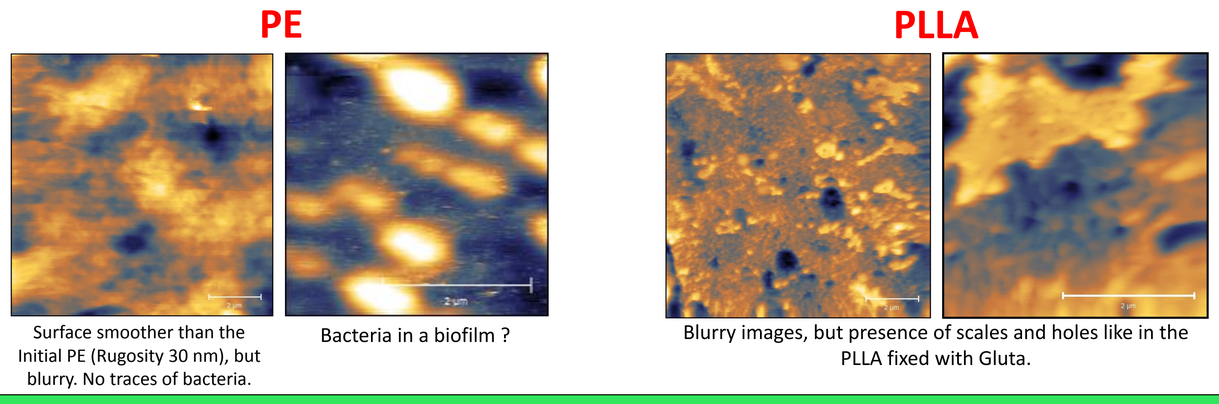
1. Experimental procedure



2. Ageing of various polymers *(fixed with Glutaraldehyde)*



3. Observations in *sterile sea water* after 199 days of ageing



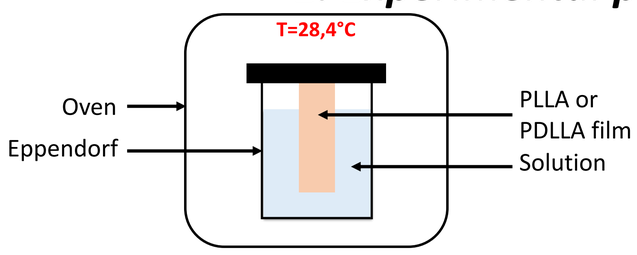
Surface smoother than the Initial PE (Rugosity 30 nm), but blurry. No traces of bacteria.

Bacteria in a biofilm ?

Blurry images, but presence of scales and holes like in the PLLA fixed with Gluta.

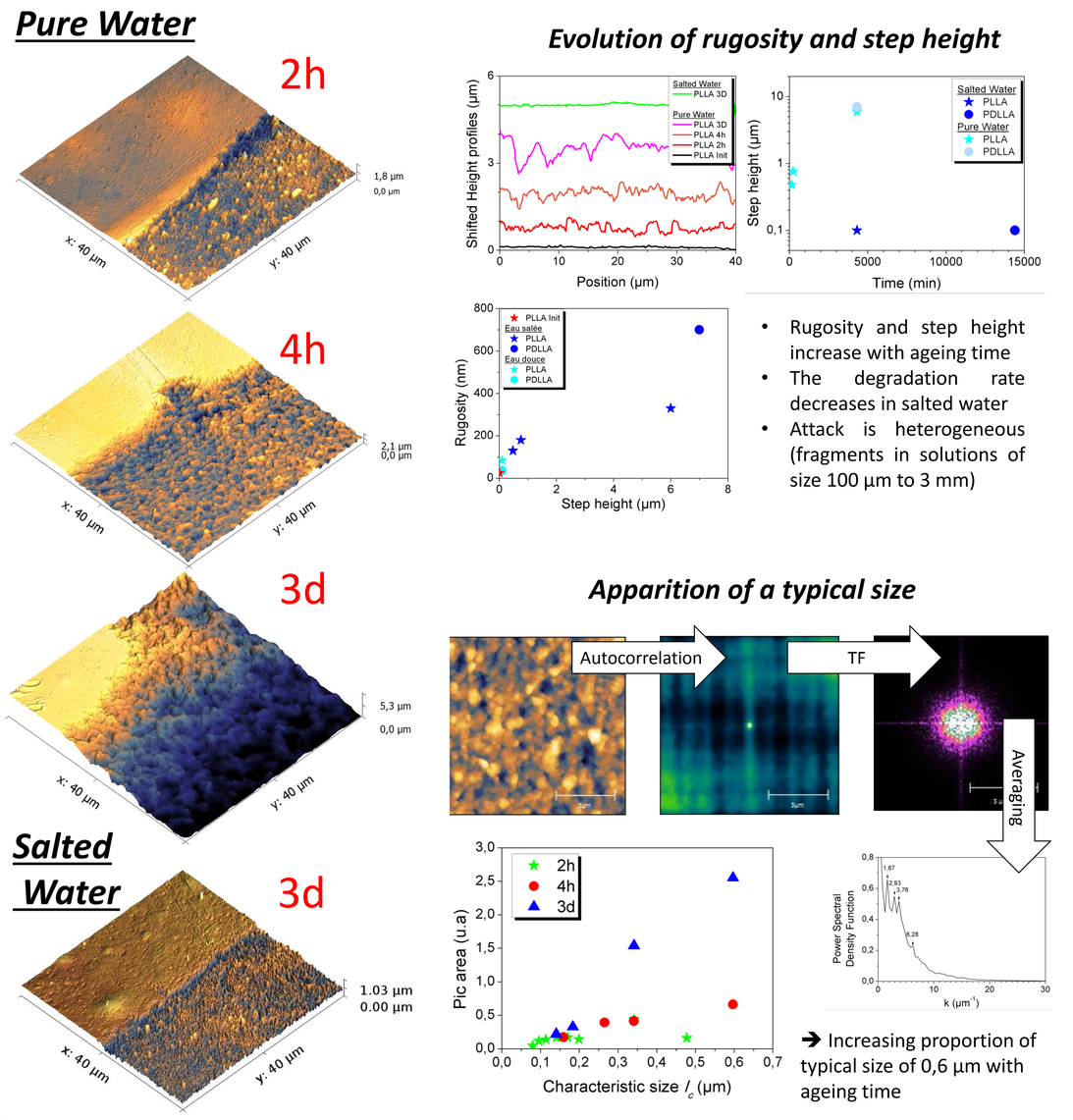
Polymer ageing in enzyme solution

1. Experimental procedure



Solution [5]:
- Tris-HCl Buffer pH 8
- Enzyme « Proteinase K » 100 µg/mL
- Pure Water OR Pure Water + **Marine salts** 35 mg/mL

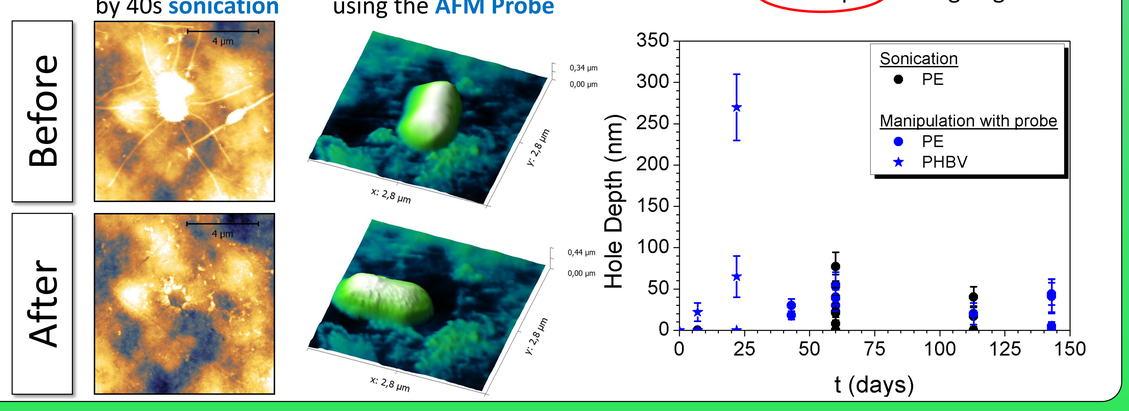
2. Ageing of PLA films



- Rugosity and step height increase with ageing time
- The degradation rate decreases in salted water
- Attack is heterogeneous (fragments in solutions of size 100 µm to 3 mm)

➔ Increasing proportion of typical size of 0,6 µm with ageing time

4. Manipulation of biological objects



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[2] Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., & Nava-Saucedo, J. E. (2008). *Chemosphere*, *73*(4), 429-442.
[3] Beech, I. B., Smith, J. R., Steele, A. A., Penegar, I., & Campbell, S. A. (2002). *Colloids and Surfaces B: Biointerfaces*, *23*(2), 231-247.
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Conclusion and Outlook

AFM was used as the main tool to study the ageing of various polymer films in marine environnement or in enzymatic solutions. In particular we followed the colonisation of the films by bacteria and the early alteration of the polymer surface. Yet numerous questions remain. More experiment using the **AFM liquid cell** are required to observe **biofilms** on polymers **in-vivo**, coupled to CR-AFM measurements. On the other hand, as one purpose is to favor the assimilation of polymers by bacteria and to avoid their fragmentation, we want to study the role of **heterogeneities** (size of crystalline domains) on the **fragmentation** of polymer films by **enzymatic** degradation.