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3D Cryo-FIB/SEM For Microalgae Filtration Applications: Probing Biomolecules Buried Inside Porous Polymeric Media

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The membrane filtration process allows to extract, concentrate, purify and separate different components of a complex mix. The main role of the membrane is to allow the permeation of one element, while blocking others because of size or charge exclusion. Recently, they have been adapted for microalgae valorisation, where filtration employing porous polymer membranes is used to separate and recover lipids and proteins from ground microalgae aqueous extracts. The biomolecules recovered can be used in pharmaceutical industry, cosmetics, food supplements or biofuel industry as biodiesel [1], [2]. During filtration, obstruction of membrane pores and accumulation of biomolecules at the surface and in the porous medium, called fouling, is a major operational challenge and a well-known drawback in membrane filtration. A detailed characterization of the pore structure, as well as its interaction with the target biomolecules, is essential to understand and help minimize the fouling of the membrane. Widely used commercial polymer membranes such as those used in this study: the PAN (polyacrylonitrile, nominal 30nm pore size, Orelis) and the PES (polyethersulfone, ominal 0.1µm pore size, Koch), have a so called asymmetric structure, which includes the presence of a very thin selective layer and changing pore structure with depth.

Generally, porous polymeric materials used as filtration membranes are characterized by SEM (Scanning electron microscopy) with direct visualization of the material structure in surface or 2D cross section [3]. However, the membrane pores, where fouling occurs, are 3D structures and require 3D characterization methods for a complete description which accounts not only for the structure of the membranes (asymmetric membranes are used) but also for the relative amount of pores that are blinded and won't go through the selective layer [4]. Polymers and biomolecules are particularly difficult to image due to the fact that they are amorphous, non-conductive, present little contrast and a high sensitivity to the electrons. The development of methods for acquisition at cryogenic conditions permit to observe fouled hydrated membranes after filtration while minimizing damage on polymer and sensitive biomolecules, such as lipids or proteins, maintaining material native structure [5]. Additionally, lipids and proteins, must be stained in order to be able to identify them properly within the polymer matrix. Notably, methods that allow to distinguish them within a complex mixtures containing both: lipids and proteins, are needed. Finally, reconstruction protocols for low contrast materials and interfaces must be applied.

In this study, Membranes were filtrated with stained lipids and proteins until fouling occurs and cryo-fixed by HPF (High pressure freezing) before it observation. In this presentation, I will show our latest results regarding the preparation, 3D acquisition and reconstruction by FIB/SEM of hydrated fouled membranes under cryogenic conditions with a 5nm voxel size. The different technical challenges for maintaining cryogenic temperatures and vitrification of the sample, optimising milling and deposition conditions, 3D reconstruction and analysis will be discussed (figure 1). The analysis of both lipids and proteins fouling inside the membrane, aided by complementary methods of high-resolution imaging and spectroscopy, as well as the fouling-membrane structure relationships will be also discussed. In perspective, the development of cryo-lamella on fouled membrane as well as the use of TEM/STEM cryo-microscopy coupled with spectroscopy (EDS and EELS) can also allow accessing to additional chemical and structural information with higher spatial resolution.

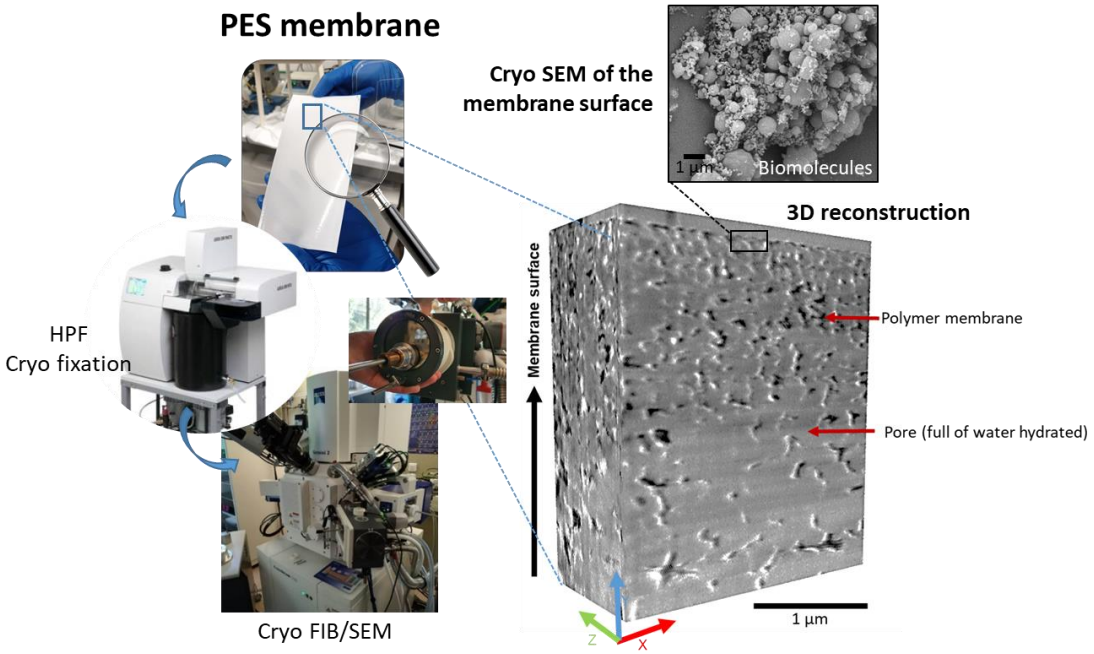

Figure 1: 3D volume reconstruction obtained by FIB/SEM for the PES membrane under cryogenic condition (hydrated membrane fouled by stained lipid). Arrows show the membrane structure and filled (hydrated) pores. Under cryogenic conditions, bright contrast could be associated with stained biomolecules (a detailed picture of biomolecules on the membrane surface is shown). Main steps of the sample preparation are presented (cryo fixation by HPF, cryo transfer, cryo FIB/SEM observation and acquisition) on the left part.

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