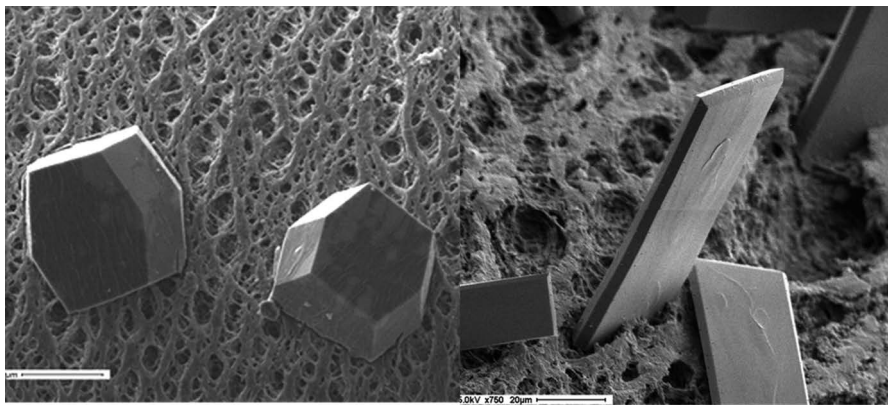

Figure 8.7 SEM image of crystals nucleated and grown on a microporous polypropylene membrane surface. (A) tetragonal lysozyme. Reprinted from ref. 30, Copyright 2003, with permission from Elsevier. (B) trypsin crystals. Reprinted with permission from ref. 10, Copyright 2005 American Chemical Society.

corresponding energy barrier in homogeneous phase is plotted against the contact angle. Points are located on the diagram according to the contact angle measured for a lysozyme solution (40 mg mL^{-1}, 2% w/v NaCl, pH 4.6) on different lab-made PVDF membranes. In general, the energetic barrier decreases at higher porosity and increases at higher contact angle; therefore, in a membrane crystallizer, nucleation rate is accelerated by highly porous and moderately hydrophobic substrates.

From a theoretical point of view, Monte Carlo simulations confirm that nucleation in a pore is always faster than on a perfectly smooth surface.[24] The scientific literature also provides empirical evidence of a favorable effect of porosity on the nucleation rate; in particular, Chayen *et al.* (2006) proved that the nucleation of thaumatin, trypsin, lobster α-crustacyanin, lysozyme, c-phycocyanin, myosin-binding protein-C, and α-actinin actin binding is enhanced when occurring in the presence of a porous medium, while nonporous surfaces are less successful in promoting nucleation.[25]

Accelerated crystallization kinetics and reduced induction time with respect to conventional crystallization techniques, while preserving diffracting quality of protein crystals, are generally observed in membrane crystallization.[28,30,31] SEM micrographs of tetragonal lysozyme and orthorhombic trypsin crystals embedded on microporous polypropylene membrane surface are shown in Figure 8.7.

8.6 Crystal Morphology and Polymorphism

The evolution of nuclei to macroscopic crystals can follow several pathways finally resulting in different product characteristics such as particle size distribution, shapes and structures (polymorphism) that strongly depend on the nucleation and the growth rates. In addition, the morphology of a crystal