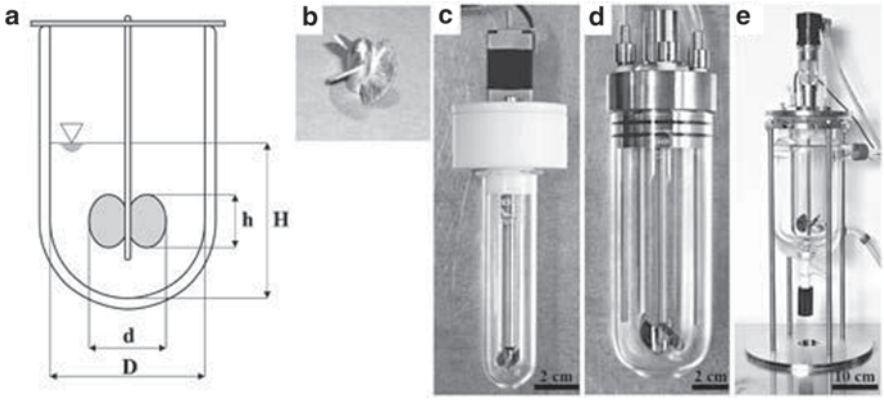

Fig. 5 Geometrical design of the crystallization systems. **a** Schematic drawing, **b** three-bladed segment impeller ($d = 11.3$ mm), **c** 6-ml vessel, **d** 100-ml vessel, and **e** 1-l vessel (Adapted with permission from [53])

of crystallization vessels and the three-bladed impeller used for scale-up development [53].

While there are several considerations and limitations with bulk protein crystallization, it offers the following distinct advantages:

1. It is a general observation that several protein instability reactions proceed slowly in the solid rather than the liquid state, hence crystallization can serve as an ideal polishing step for final processing of biologics wherein water is eliminated or minimized from the system and material is converted into a more stable solid state. In case of insulin, bulk crystallization followed by freeze-drying is commercially adopted to ensure stability of the material for pharmaceutical distribution and long-term storage before formulation.
2. Bulk lyophilization of protein solutions is a rather energy consuming step and can often be deleterious to sensitive proteins (aggregation or protein degradation). In such instances, it becomes practical to crystallize/precipitate the protein and then remove the residual water from the suspension by bulk freeze-drying. The combined application of crystallization and freeze-drying as subsequent unit operations becomes an economically viable option for large-scale biopharmaceutical manufacture.
3. Bulk crystallization, a relatively inexpensive technique, can replace cost-intensive standard biopharmaceutical unit operations like packed-bed and membrane chromatography. Przybycien et al. present a comprehensive review of bioseparation operations wherein they compare alternative bioprocess techniques to standard packed-bed chromatography (Fig. 6) [43]. Crystallization happens to be a relatively mature industrial processing technique with several applications. Several studies describe successful purification and polishing of proteins such as aprotinin [42], mAbs [17, 52], and lysozyme [19, 24, 25] by bulk crystallography approach.