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X-ray powder diffraction: A powerful tool for industrial protein production

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X-ray powder diffraction (XRPD) offers a method of characterizing a crystalline protein suspension [1], and data can be collected within 30 minutes, which is appealing for industrial applications. In industry, enzymes are produced and handled in high concentrations, which can in turn cause problems for the processes due to protein precipitation in the production pipeline. XRPD is useful for identification of the crystal forms present, by fitting calculated patterns of known single crystal forms to the observed XRPD pattern. For this purpose we have developed a streamlined program for calculation of diffraction patterns from pdb-files taking into account bulk-solvent, peak asymmetry and background [2]. XRPD was applied to a suspension from a large-scale industrial production of the widely used Bacillus lentus subtilisin. A dominant crystal form was identified by XRPD, but two other different crystal forms were found by a complementary single crystal micro-diffraction analysis of the larger single crystals present in the sample [3]. The study serves as a reminder that when a crystal is picked out from a batch crystallization for single crystal analysis, it might not be representative of the bulk microcrystalline material in the sample. To estimate the fraction of the different crystal forms in production samples with significant polymorphism, a further XRPD study was performed on binary mixtures of different lysozyme and subtilisin crystal forms. Quantitative XRPD generally requires careful sample preparation, and working with protein slurries leads to further challenges in terms of varying crystal density. After careful optimisation of suspension medium, the relative composition of crystal forms can be determined within 10%. This work demonstrates the value of in-house XRPD as an analysis tool in industrial enzyme production, and its potential to help troubleshooting the production process and to provide information for further refining the manufacturing of enzymes.

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