Optical Methods and Their Limitation to Characterize the Morphology and Granulometry of Complex Shape Biological Materials

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Background and aim: Particle size and morphology of biomass (microorganism, lignocellulosic substrates) stand out as the major determinants of the bioprocess efficiency. Through its impact on rheology, it affects momentum, heat and and mass transfers within the bioreactor. Various techniques are available to characterize in-situ and ex-situ size and shape of particles. The most common methods are classified into three groups: (i) analysis of microscopic images; (ii) laser light diffraction and (iii) settling kinetics. In present work, five techniques are compared and discussed with model particles, microorganisms and lignocellulosic substrates.

Methods: The used techniques aim to characterize size and shape (0.1 to 2000µm). In-situ and ex-situ measurements were used: chord length measurement (FBRM), diffraction light scattering (DLS), morpho-granulometry (MG), cytometry (CYT) and settling velocity (TUL). A set of height polystyrene microspheres (1.0 to 15.0µm) and microbeads (40 and 80µm) were used as reference. *Yarrowia lipolytica* is strictly aerobic yeast, belonging to the family of hemiascomycetes. Cells are subjected to mycelial transition induced by pH changes. Its morphology evolves from ovoid shape (5-7µm) up to filament. It was used to appreciate the ability to qualify and quantify filamentous shape (width, length). Finally, two cellulosic matrices, microcrystalline cellulose and coniferous paper pulp were selected to investigate complex fiber morphologies.

Results: Specifications and limits of instruments are scrutinized. Sampling methods and preparation should be carefully considered. Optical measurements provide raw data (light intensity, frequency, images) from which morphological parameters will be straightly extracted or calculated based on assumptions (optical properties, particles geometry, theory). Considering diameters and associated number and volume distribution functions, techniques are compared with model calibrated microspheres. The mean values appear consistent between techniques but the magnitude of standard deviation extensively varies. Few instruments (MG, CYT) provide access to additional morphological criteria (length, width, aspect ratio). Mycelial kinetics and magnitude is accurately described by fiber length (MG). However a poor reliability of width (time of flight, CYT) is noticeable. Considering more complex lignocellulosic particles, the relative diameter values usually indicate similar trends whatever the techniques is. However, absolute values should be carefully considered and may deviated in large extend (5-10 times).

Keywords: granulometry, morphology, spherical particles, filamentous microorganism, lignocellulosic substrates, CLD, PSD, focus beam reflectance, diffraction laser light scattering, cytometry, microscopy, settling kinetics