

## Abstract Submission

Submission-2649

Topic : / Biochemical Engineering / ECAB3 / Bioproducts (or bio-based products)

### COMPARISON OF OPTICAL METHODS TO CHARACTERIZE MORPHOLOGY AND SIZE DISTRIBUTION OF MODEL PARTICLES AND MYCELIAL TRANSITION OF *YARROWIA LIPOLYTICA*

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Authors of the abstract come from :: Both University and Industry

**Submit your abstract below (400 words):** During cell culture in bioreactor, bio-performances (physiology and activity) are strongly affected by flow, mass and heat transfer limitations. Amongst the main parameters to be studied, the rheological behaviours of the suspension and cell morphology stand out as the major physical determinants of the process efficiency. They are the principal elements for the choice of the equipment and the culture strategy. This survey details and compares different optical methods used to characterize particle size and shape. Four ex-situ methods were compared: (i) diffraction light scattering (Mastersizer 2000X), (ii) morpho-granulometry (Morphology G3S), (iii) flux cytometry (BD Accuri C6) and (iv) decantation kinetics (Turbiscan lab). The objectives were firstly, to develop and to validate complementary methodologies with calibrated microsphere particles (single and mixed populations – Polystyrene beads / Molecular probe) and secondly, to quantify and to characterize the morphological evolution of *Yarrowia lipolytica* W29 during an axenic oxidative culture (28°C, pH7, 1.6L bioreactor, pO<sub>2</sub>>10%, Mixing rate: 200 to 800 rpm, Air flow: 5 to 30 NI/h) in batch mode with a mineral medium and a carbon source (glucose 10g/L).

To compare these methods, the particle sizes (Fig 1) of various matrices and to monitor their changes during bioreaction, the analysis of distribution profile is preferentially used. These distribution profiles can be compared via distribution functions, E(x) and cumulative, F(x) expressed in number, length, surface or volume. Each distribution function can be characterized by a range of moment and centred moments of order j.

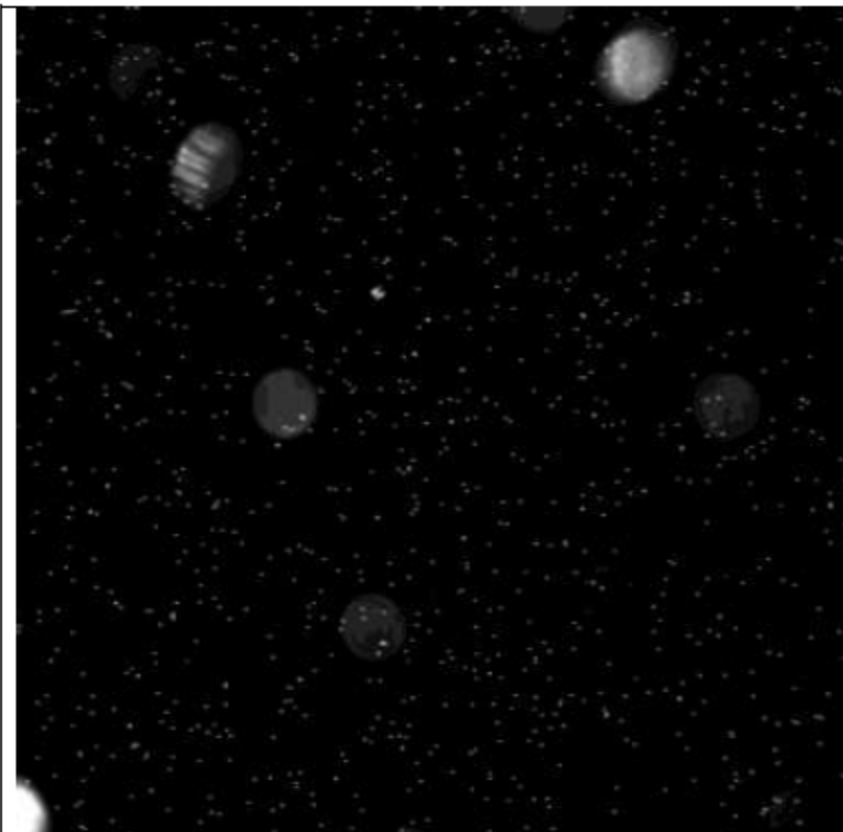
Our results highlight and compare precision, pertinence and divergence of these methods. These techniques allow to characterize the particle size distribution, to discriminate close (4-6µm) and different size (1-15µm) of mixed bead populations, and to quantify each sub-population. However size dispersion may significantly differ between techniques (Fig 2). Morphogranulometry, diffraction light scattering and cytometry were used to characterize and to quantify mycelial transition of *Yarrowia lipolytica*. During the batch culture at pH7, the proportion of cells with dce (Circle equivalent diameter)>10µm, quantified by morphogranulometry, grows from 14 / 32% (in number/in volume) for inoculum to 25 / 73% (in number/in volume) after 13h culture (6gdcw/L). Meantime, aspect ratio (AR) evolves from 0.58 / 0.53 (in number/in volume) up to 0.46 / 0.30 (in number/in volume). Even if mycelial transition is noticeable, methods may conduct to ambiguous trends and interpretation. Consequently morphological parameters are discussed.

Fig 1: Images from morphogranulometry, polystyrene microspheres (DF, 1x1mm<sup>2</sup>, x20), (A) 1µm and (B) 15µm and yeast cell (DF, 1x1mm<sup>2</sup>, x10), (C) inoculum and (D) end of culture.

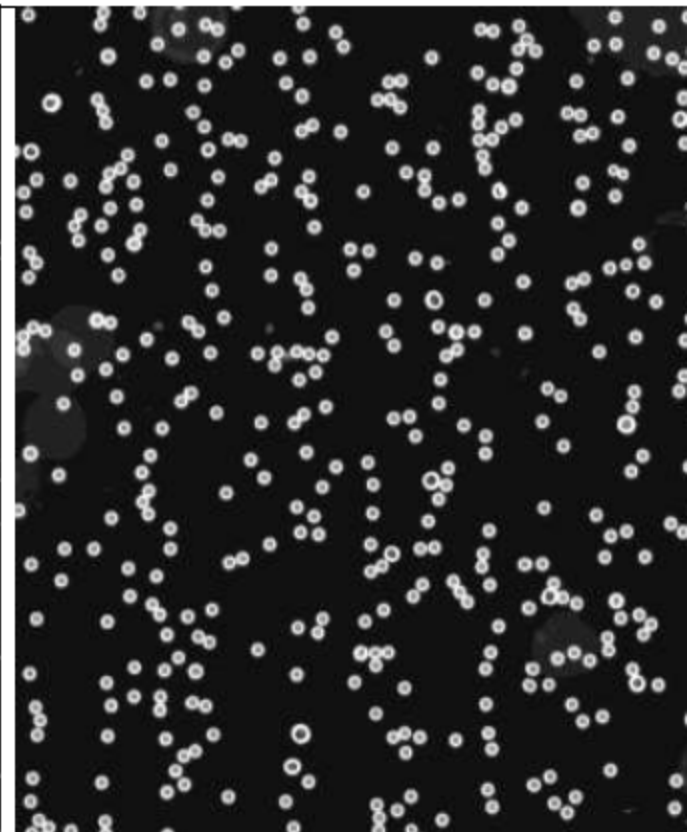
Fig 2: Distribution curves issued from diffraction light scattering, morphogranulometry and cytometry (polystyrene microspheres 15µm).

Type of presentation :: Oral

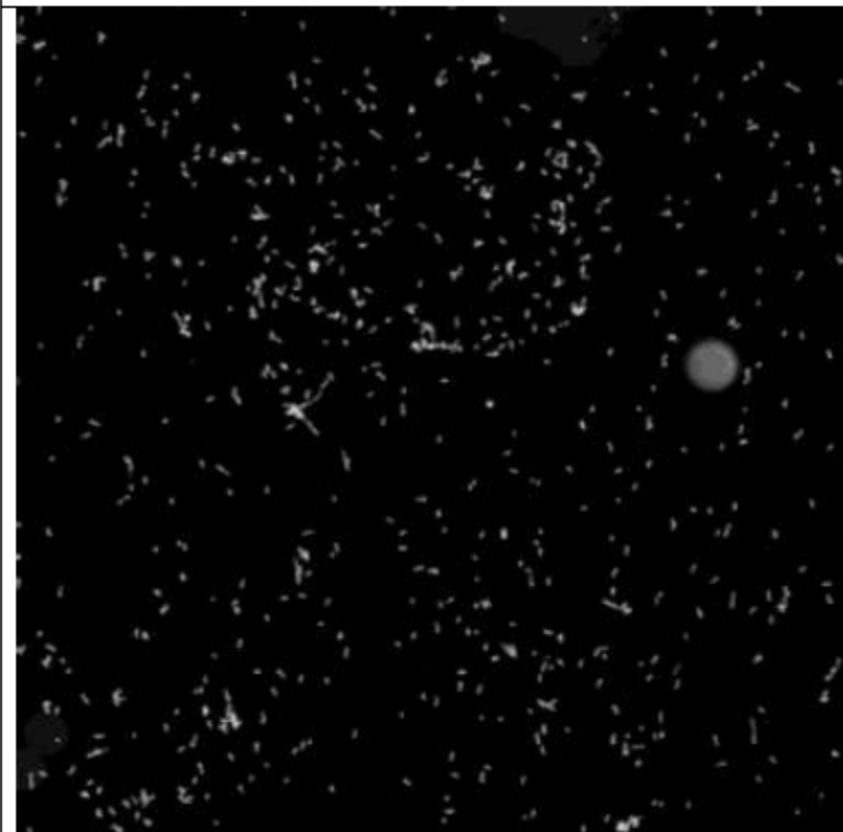
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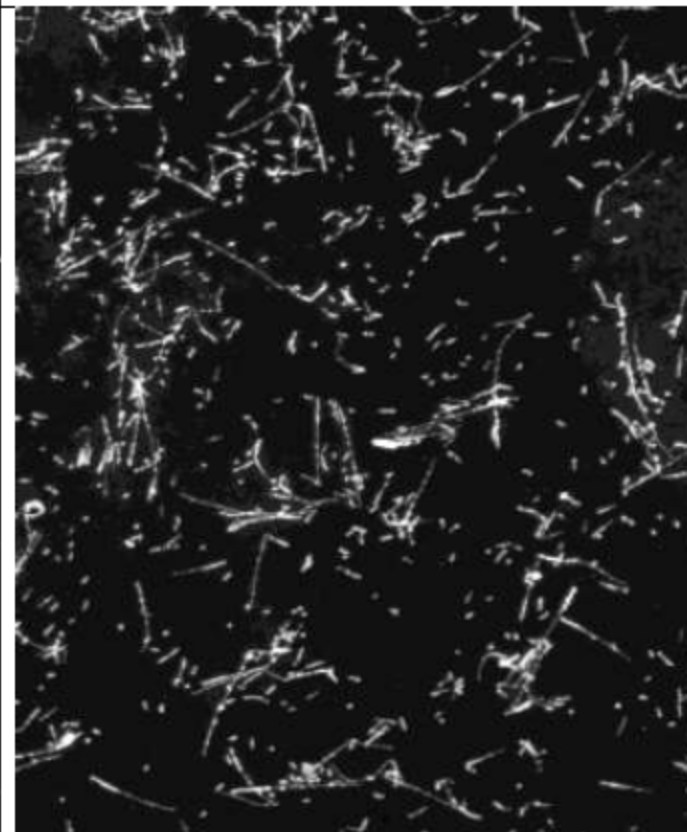
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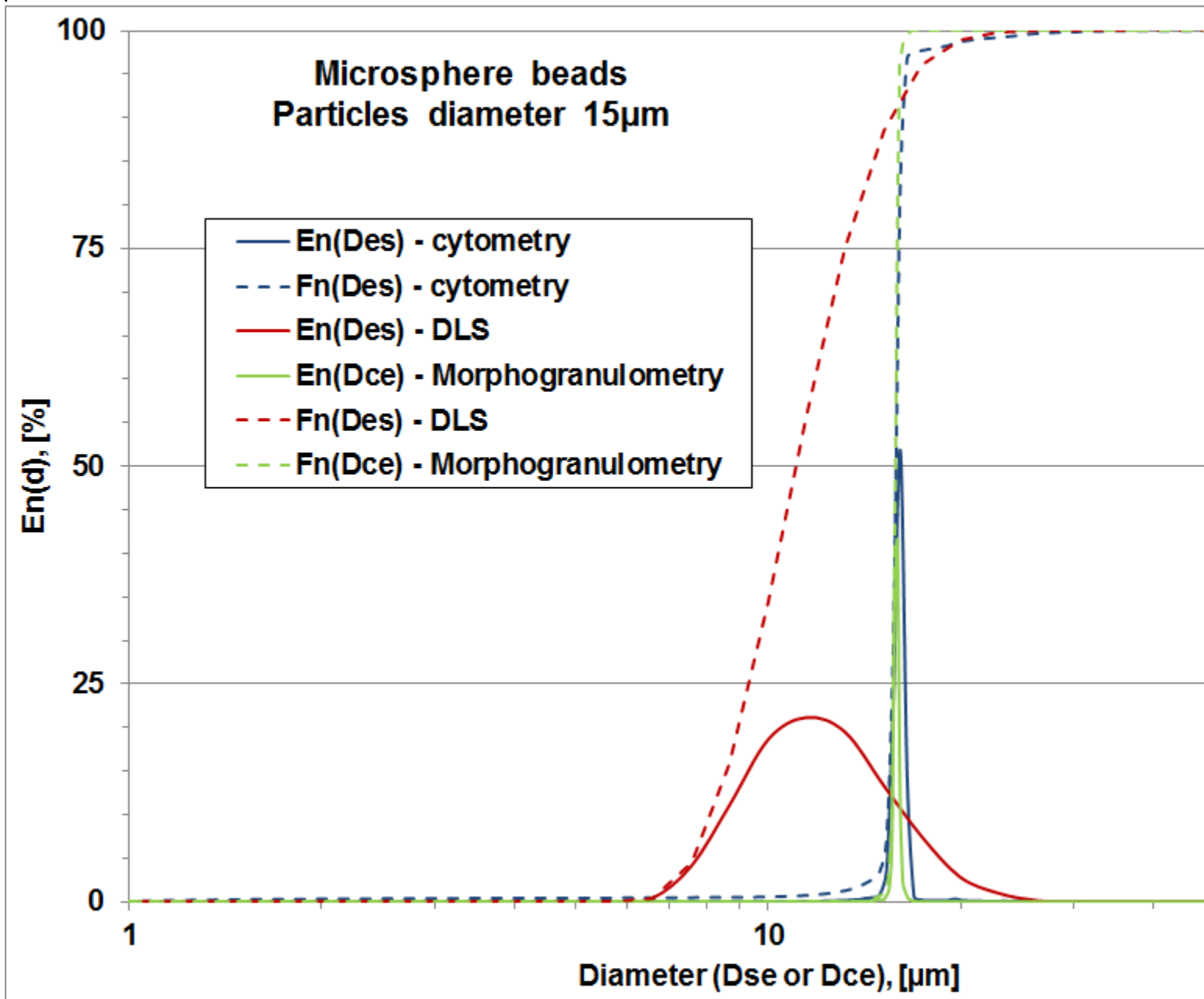
B



C



D



**Highlight 1:** ex-situ optical methods to characterize particle size distribution in bioprocess

**Highlight 2:** statistical comparison of distribution function is achieved

**Highlight 3:** morphogranulometry appears as the most reliable methods

**Keywords:** Bioreactor, Experimental methods, Fermentation, Yeast