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Characterization of mycelial transition of *Yarrowia lipolytica* during oxidative cultures: comparison of optical methods and their limitation, impact of morphology on rheological behavior.

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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 mass transfers within the bioreactor. Consequently, the rheological behaviors of the suspension and cell morphology stand out as the major physical determinants of the process efficiency and are the principal elements for the choice of the equipment and the culture strategy.

This presentation will illustrate and compare *in-* and *ex-situ* methods to qualify and to quantify the mycelial transition of microbial cell and the rheological behavior of broth during axenic oxidative cultures with *Yarrowia lipolytica* as model strain.

Methods: Considering morphogranulometry, 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 (in-situ chord length measurement FBRM, diffraction light scattering DLS, morpho-granulometry MG, cytometry CYT and settling velocity TUL) to characterize size and shape (0.1 to 2000 μ m) are compared and discussed: A dedicated methodology was developed to qualify and quantify objectively the morphological changes of cell population.

Considering rheometry, classical *ex-situ* measurements were carried out (rheometer Mars3, cone-plane device 60mm/1°) after sampling. *In-situ* rheometry rests on an original pilot based on a bioreactor (20L) with a derivation loop including a specific on-line rheometric device as well as additional physical and biological measurements. Through pressure drop and flowrate measurements, knowing friction curves of calibrated ducts and considering Metzner&Reed (1953) concept, the viscosity can be estimated in respect with accurate and stringent conditions imposed by cell culture strategy.

Strategy: *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 (5-7 μ m) up to filament. This strain was used to appreciate the ability to qualify and quantify filamentous shape (width, length) and its impact on rheological behaviour. Cell culture under well controlled conditions (standardized inoculum, axenic oxidative culture, mineral medium, glucose as carbon source) were



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conducted at various pH (4.5, 5.6 and 7) under batch and fed-batch modes were used. In batch mode ($V=2L$, $0.1 < [X] < 5 \text{ gcdw/L}$), the impact of pH on mycelial transition enable to validate morphogranulometric methods and data treatment. In fed-batch mode ($V=15L$, $0.1 < [X] < 100 \text{ gcdw/L}$), the impact of mycelial transition on rheological behavior was scrutinized at pH5.6 (ovoid shape) and pH7 (filamentous shape) though *in-situ* and *ex-situ* rheological measurements.

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). 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) and cylinder model. In batch cultures, different pH conditions (4.5, 5.6 and 7) were investigated in order to identify the pH inducing a stress response in *Y. lipolytica*. Macroscopic behavior (kinetic parameters, yields, viability) of the yeast was slightly affected by the pH of the culture. However, contrary to the culture at pH 5.6, a predominant filamentous growth was induced in batch experiments at pH 4.5 and pH 7. Proportions of the filamentous subpopulation reached 84% and 93% (v/v) under acidic and neutral conditions, respectively. In fed batch, physical properties of broth were investigated versus cell concentrations (0.1 to 100 gCDW/L) with growth rate ($\mu=0.1$ and 0.2 h^{-1} , #1 and #2) and a dimorphism generation by pH changes (pH=5.5 to 7, $\mu=0.1 \text{ h}^{-1}$, #3). Rheological behavior switches from Newtonian to Non-Newtonian power-law behavior at cell concentration threshold ($\sim 10 \text{ gcdw/L}$). Threshold transition and viscosity increase (x10) is observed even with low proportion of filamentous cells.

Keywords: granulometry, morphology, rheometry, filamentous microorganism, CLD, PSD, focus beam reflectance, diffraction laser light scattering, cytometry, microscopy, settling kinetics.

Short-CV: Fillaudeau Luc (47 year old) is INRA scientist since 1999 and works in laboratory for Biosystems and Chemical Engineering LISBP (specialized in biotechnology, system biology and chemical engineering). His activity is focused on Bioprocess and Food engineering. His research field is dedicated to the study of coupled transfers and transfer limitation in food and bioprocess. It is described by 3 keywords: Cell cultures (polyphasic and reactive medium), Bioreactor, Physical characterization. His expertise is related to food processing/safety and bioprocess engineering. His scientific production (5 patents, 45 publications, 150 communications, 9 chapters) deals with membrane process, heat treatment, bioreaction and complex biological matrices.