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In-situ and ex-situ rheometry of high density *Yarrowia lipolytica* broth: determination of critical concentration and impact of yeast-mycelial transition

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Abstract : The specificity of microbial bioreactions which give rise to irreducible couplings with hydrodynamics and heat and mass transfers, led into complex (three phases medium) and dynamic (auto-biocatalytic reaction) systems. Cells (concentration, shape, dimension, physiology...) strongly affect physico-chemical properties of broth and the modification of these characteristics interacts with bioprocess performances (specific rates, yields...) with an improvement or, more generally, a decrease of yields.

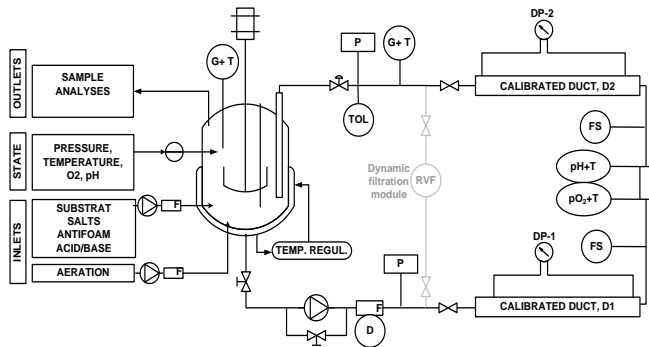


Fig. 1: Overview of experimental set-up

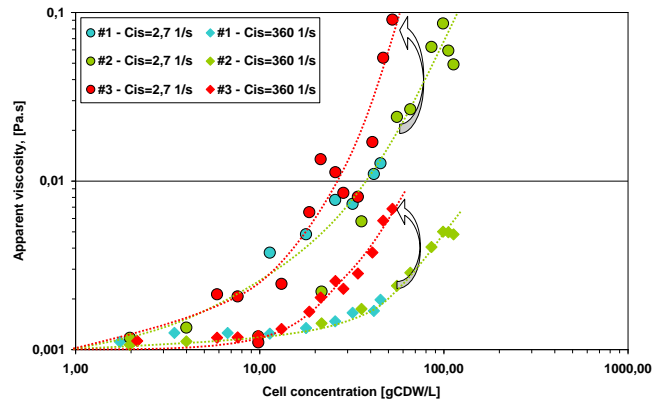


Fig. 2: Evolution of apparent viscosity (cell broth) as a function of cell concentration and impact of mycelial transition on rheological behaviour.

Our approach rests on physical and physico-chemical on-line and off-line measurements in respect with accurate and stringent conditions imposed by cell culture strategy. 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 is used. Axenic cultures of eucaryote microorganisms (*Y. lipolytica*) under pure oxidative fed-batch conditions are considered in order to evaluate biomass effect (concentration, morphology). Growth rate is controlled by carbon feed under synthetic mineral medium. Bioperformances related to oxygen transfer and physical properties of broth (in-situ and ex-situ rheometry) versus cell concentrations (0.1 to 110 gCDW/L), growth rate (cultures #1 and #2, $\mu=0.1$ to $0.2h^{-1}$) and dimorphism generation (cultures #3, pH=5.5 to 7, $\mu=0.1h^{-1}$ for $X>5gCDW/L$) are considered. Cell morphology is scrutinized along fed-batch cultures. In-situ and ex-situ rheometry are consistent and complementary analysis indicating a shear-thinning behaviour. Critical cell concentrations are observed and a significant deviation of rheological behaviour is attributed to yeast-mycelial transition. A structured rheological model is established considering cell concentration (volume fraction), population balance and shear rate.