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IRREDUCIBLE COUPLING BETWEEN PHYSICAL AND BIOLOGICAL PHENOMENA: OVERVIEW OF ON-LINE AND OFF-LINE PHYSICAL MEASUREMENTS DURING HIGH CELL DENSITY CULTURES OF YARROWIA LIPOLYTEICA.

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During cell cultures in bioreactor, micro-organism physiology closely interacts with physico-chemical parameters (gas and feed flow rates, mixing, temperature, pH, pressure). The specificity of microbial bioreactions in relation with irreducible couplings between heat and mass transfers and fluid mechanics, led into complex (three phases medium) and dynamic (auto-biocatalytic reaction) systems. Our scientific approach aims to investigate, understand and control dynamic interactions between physical and biological systems at different scales (macro, micro and molecular) for molecules, strains and/or bioprocess innovation in a white biotechnology context. 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. Among these properties, rheological behaviour is a strategy widely used to understand the impact of cells and the modification of bioprocess performances.

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. This work leads to design and realise 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. Y. lipolytica cultures were investigated with a control of growth rate by carbon feed within concentrations ranging from 0.1 up to 60gCDW/L. On-line and off-line measurements are discussed regarding similar or opposite tendencies along culture. If the off-line density could be correlated with cell concentration, on-line measurement exhibit opposite tendancy. On-line and off-line rheological measurements are consistent. Results are discussed in terms of size, morphology, surface properties, concentration, biological activity and compared to scientific literature. On-line rheology highlight about the Non-newtonian rheological behaviour of broths and the gap between on-line and off-line measurements.

Key-words: broth, cell culture, fed-batch, on-line and off-line measurement, physical and physicochemical properties, rheometry, Escherichia coli, Yarrowia lipolytica.