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# IMPACT OF CELL PHYSIOLOGY AND DENSITIES DURING OXIDATIVE AXENIC CULTURES OF *YARROWIA LIPOLYTICA* ON PHYSICO-CHEMICAL PROPERTIES OF BROTH

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## 1. INTRODUCTION

Accurate measurements of cell properties (concentration, shape, dimension, density) are fundamental to understand microorganism physiology and they strongly affect physico-chemical properties of broth. These characteristics interact with bioprocess performances (specific rates, yields...) with an improvement or, more generally, a decrease of yields. Experiments were conducted with a singular set-up based on a bioreactor (20L) with a derivation loop including in-situ physical and biological measurements (viscosity, specific density, mass flow rate, electrical conductivity, pH, pO<sub>2</sub> and temperature). Three axenic cultures with *Y. Lipolytica* were investigated in pure oxidative culture in order to produce biomass up to high cell concentration with a control of growth rate by carbon feed (synthetic mineral medium). Cell size and morphology, intracellular water and salts contents are scrutinized along fed-batch cultures. Our strategy aims to investigate the evolution of bioperformance related to physical properties of broth (rheometry, densimetry, conductimetry, cell morphology) versus cell concentration (ranging from 0.1 to 110 gCDW/L), growth rate ( $\mu=0,1$  to  $0,2$  h<sup>-1</sup>, cultures #1 and #2) and dimorphism generation by pH changes (pH=5,5 to 7;  $\mu=0,1$  h<sup>-1</sup>; culture #3).

## 2. MATERIALS AND METHODS

### 2.1 *Experimental set-up and instrumentation*

The experimental set-up, described by Manon et al. 2011, consisted of a 20 L bioreactor (Chemap-Fermenter, Chemap AG, CH-8601 Volketswil), a displacement pump (TUTHILL DSG 1.3EEET, Q<sub>max</sub>: 350 l h<sup>-1</sup>, P<sub>max</sub>: 5 bars) and a fully instrumented derivation loop (Fig.1).

### 2.2 *Specific analyses*

Thermogravimetry allowed a quick and accurate estimation of intra- and extracellular water of yeast cells, dry matter, volume and density of a single cell (Illmer et al, 1999). The assumption of the method is that drying of microbial cells occurs during two phases. First, the more volatile extracellular water is lost. Not before this fraction is completely evaporated, the intracellular water starts to vaporise. A 15 ml culture broth is sampled, centrifuged (10min, 4800 tr/min, 4°C), supernatant is removed, weight are controlled at each step. The cell sediment is then diluted with a known weight of distilled water (2/3 of sediment



Cell physiology was investigated along cultures through microscopic observation (Fig 2), laser granulometry and morphogranulometric measurements. For the first two cultures (#1, #2), an extension of cell was observed at the beginning of the culture, but these morphological changes are negligible comparing to the dimorphism generation during the culture #3. In initial step, a reduction of mean diameter and sharp decrease of size span was observed. This phase correspond to the adaptation of inoculum to new culture conditions and physico-chemical environment. Over 3gcdw/L, cell populations became homogeneous (#1 and #2) with a mean diameter close to 5 $\mu$ m. In culture #3, over 5-10gcdw/L a pH change was imposed to induce a mycelial transition. An increase of population fraction whose diameter is greater than 10 $\mu$ m was observed, which grows from 1.5 up to 12%vol/vol (Fig. 3).

Table 1: Performances overview and cell morphology

Cultures	#1	#2	#3
Growth rate, $\mu$ [h <sup>-1</sup> ]	0.180	0.085	0.09 and 0.075 (stress)
Cell concentration, X [gms/L]	0.46 $\rightarrow$ 63	0.42 $\rightarrow$ 114	0.38 $\rightarrow$ 4.7 $\rightarrow$ 56
Shape	Ovoïdal	Ovoïdal	Ovoïdal/Filamenteous
Size D[4,3] (D0.1, D0.9) [ $\mu$ m]	5.0 (2.1/9.4)	3.8 (2.1/5.9)	4.5 (2.1/7.6)

Surface measurements (surface diameter,  $d_s$  or equivalent circle diameter) obtained by morphogranulometry laser were performed for culture #3. In accordance with the laser granulometry, a slight decrease of equivalent circle diameter was observed and reflects the initial state of the inoculums.

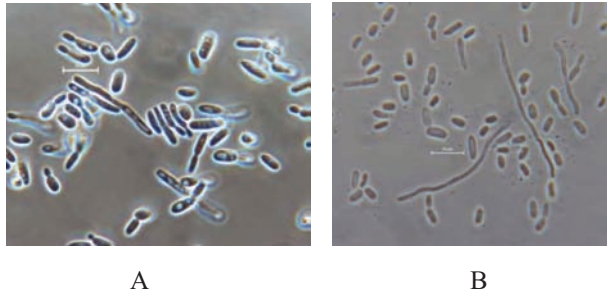


Fig.2. Cell observations (x100, oil, 10gcdw/L) for cultures #1 (A) and #3 (B).

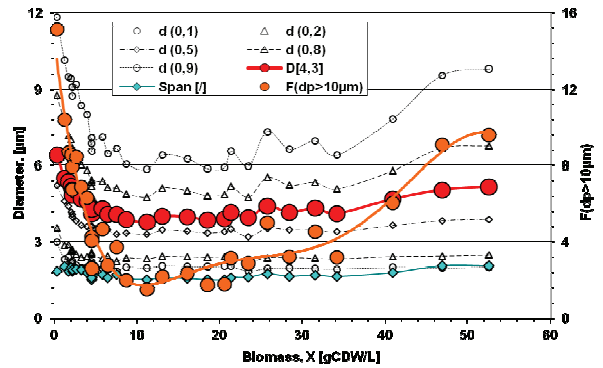


Fig. 3. Diameters evolution  $d(0.1)$ ,  $d(0.5)$ ,  $d(0.9)$ ,  $D[4.3]$ , dispersion (span) and Fraction  $F(dp > 10\mu\text{m})$  versus biomass (Culture #3)

### 3.2 Intrinsic cell density

The analysis of drying curve (Fig. 4) leads to deduce the absolute and relative water contents of biomass,  $n$  and  $x$ , along cultures #1, 2 and 3. The relative humidity of biomass ranges between 80 and 70% (equivalent to an absolute humidity between 2.3 and 4) and substantially decreases with increasing concentration. Average values of absolute and relative humidities respectively equal to 73% ( $\pm 4$ ) and 2.80 ( $\pm 0.58$ ) can be taken as a reference for *Yarrowia lipolytica*.

The mass and volume balances lead to establish relations between water content and density:

$$\text{Density: } \rho_{\text{Ex-situ}}^{\text{mout}} = \frac{m_{\text{Total}}}{V_{\text{Total}}}, \rho_{\text{XH}} = \frac{m_{\text{XH}}}{V_{\text{C}}} \text{ and } \rho_{\text{X}} = \frac{m_{\text{X}}}{V_{\text{C}}} \text{ with } x = \frac{m_{\text{eau-X}}}{m_{\text{XH}}} \text{ and } n = \frac{x}{1-x}$$

with  $\rho_{\text{X}} = \rho_{\text{XH}} \cdot (1-x)$  et  $m_{\text{X}} = X \cdot V_{\text{Total}}$

$$\text{With: } \rho_{\text{Ex-situ}}^{\text{mout}} = \rho_{\text{S}} + X \cdot \left( n + 1 - \frac{\rho_{\text{S}}}{\rho_{\text{X}}} \right) = \rho_{\text{S}} + X \cdot \left( \frac{1}{1-x} - \frac{\rho_{\text{S}}}{\rho_{\text{X}}} \right)$$

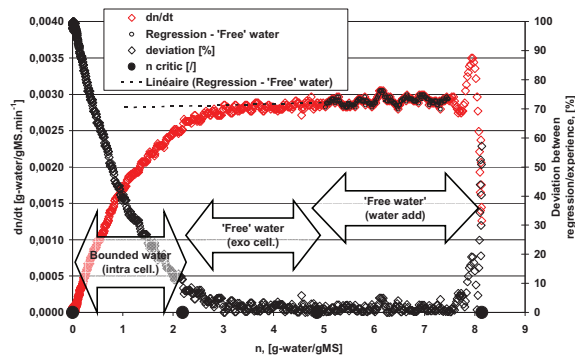


Fig. 4. Example of drying curve— Culture #3, E29

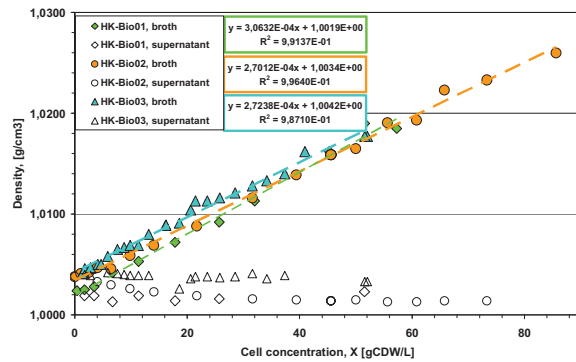


Fig. 5. Broth and supernatant ex-situ density evolution versus cell concentration - Cultures # 1, 2 and 3.

The ex-situ (broth) density will proportionally increase as a function of cell concentration,  $X$ . Slope enables to determine intrinsic cell density assuming a constant water content, origin correspond to supernatant density (Fig. 5). The ex-situ density of supernatants remains constant for cultures #1 ( $1.0017\text{g/mL} \pm 0.0003$ ), #2 ( $1.0019\text{g/mL} \pm 0.0007$ ) and #3 ( $1.0037\text{g/mL} \pm 0.0003$ ). The absence of drift of the supernatant density confirms the lack of cell lysis or co-products in the cultures. The broth density evolves linearly with the biomass concentration in agreement with theory. The intrinsic densities of biomass (assuming:  $x=0.73\text{g-water/gXH}$ ) were  $\rho_{XH}=1092\text{g/L}\phi$  ( $\rho_X=294\text{gms/L}\phi$ ),  $\rho_{XH}=1081\text{g/L}\phi$  ( $\rho_X=292\text{gms/L}\phi$ ) and  $\rho_{XH}=1083\text{g/L}\phi$  ( $\rho_X=292\text{gms/L}\phi$ ) for culture #1, #2 and #3 respectively. Wet and dry biomass densities,  $\rho_{XH}$  and  $\rho_X$ , are close to values reported for *Saccharomyces cerevisiae* by Moll (1994) and Bryan et al. (2010). However, data reported by Jarzebski et al (1989) for dry density will correspondant to other estimated wet density assuming  $n=0.73\text{ g-water/gXH}$ . For *Yarrowia lipolytica*, the mean values of wet and dry biomass densities are estimated to be  $1085\text{g/L}\phi$  and  $293\text{gms/L}\phi$  respectively. It seems that mycelial transition induced in culture #3 does not affect the biomass density.

#### 4. CONCLUSION

In-situ and ex-situ density (broth, supernatant) exhibit opposite tendency. Ex-situ density increases and is correlated with cell concentration considering population balance (size, water and salt contents). Intrinsic cell density of *Yarrowia lipolytica* has been properly identified and compared to litterature. Yeast mycelial transition (morphological change) does not affect intrinsic cell density. On the opposite, in-situ density decreases along fed-bath culture and could be significantly affected by cell physiology (oxygen consumption specific rate) and cell concentration.

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