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ON -LINE RHEOLOGY OF CELL CULTURES IN A BIOREACTOR

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Abstract: Cellular cultures require an in-depth knowledge of biological and physical parameters to control and optimize the process. Among the physical parameters, viscosity and rheological behaviour are of first importance. This study describes implementation and results obtained with an experimental on-line rheological device mounted on a bioreactor. Description of the set-up and experimental calibration with well-defined Newtonian fluids are presented. An example of a cellular culture (*E. coli*) is then proposed enlightening the influence of biological activity on rheological behaviour and the need for on-line measurement.

Keywords: cell culture, bioreactor, viscosity, rheology, on-line measurement, *Escherichia coli*.

1. INTRODUCTION

During cell culture in bioreactor, physical parameters (aeration, mixing, temperature, pH, feeds) and micro-organism physiology and activity closely interact and evolve. Irreducible couplings between heat transfer, mass transfer and fluid mechanics result in a complex and evolving system (Cascaval et al, 2003). Rheological behaviour of culture broth stands as a fundamental parameter in bioprocess performances because it affects simultaneously heat and mass transfer as well as flow pattern. Then, the understanding of rheological behaviour is determinant to drive cell culture up to a defined goal (biomass production, extra or intra cellular metabolite production, substrate biodegradation, etc.) and to optimize bioprocess (Pamboukian and Facciotti, 2005, Petersen et al., 2008).

In this context, our scientific and technical objectives are to develop and identify an experimental tool enabling on-line rheology and to validate measurements with a cell culture. To do that a bioreactor was equipped with a derivation loop which includes a specific on-line rheometric device. In a first time, the hydrodynamic identification of the loop was achieved with Newtonian model fluids. In a second time, we present results obtained during cell culture (*E. coli*, 40 to 110gCDW/L). Comparisons between on-line and off-line rheological measurements are proposed and the impact of the biological activity on the rheological behaviour of the fermentation broth is discussed.

2. MATERIALS and METHODS

2.1 *Experimental set-up*

The experimental set-up consisted of a 20L bioreactor (Chemap-Fermenter, Chemap AG, CH-8601 Volketswil), a displacement pump (TUTHILL DSG 1.3EEET) and a fully instrumented derivation loop. From a hydraulic point of view, the derivation loop consisted of a tube of 2.50m length including a 300mm hydrodynamic developing length and a pressure drop measurement along 2.00m. Smooth tubes were regular circular straight tubes (Stainless steel 316L) with a nominal diameter of 6mm and 1mm thickness. Other connections (90° junction, Te, etc) were tubes with 12mm nominal diameter. All sensors or hydraulic parts used mini or micro-clamp connections with EPDM gaskets.

Experimental measurements along derivation loop were: relative and differential pressures, mass flow rate, temperature, specific density, pH, dissolved oxygen and electrical conductivity. The relative pressure (BOURBON-HAENNI E913 33 B22 n°6008, $\pm 0.2\%$ full scale) was measured at pump outlet. A Coriolis effect flow-meter (KROHNE, type MFS-7050-S06) enabled mass flow rate (precision $\pm 0.1\%$ for a liquid and $\pm 0.5\%$ for a gas), temperature (precision $\pm 1^\circ\text{C}$) and specific density (precision $\pm 2\text{kg}\cdot\text{m}^{-3}$) measurements. The differential pressure (HONEYWELL - STD 120 n°0630-C2856562001002, precision $\pm 0.003\%$ for 10^5Pa in full scale) was used to determine the pressure drop along the calibrated length. pH and dissolved oxygen were measured with two specific sensors (pH : Easyferm Plus VP/120-238 633, pO_2 : Oxyferm FDA/120-237 450). Finally, electrical conductivity and temperature were controlled by an electrical conductimeter (KEMOTRON - type 9147 n°36036 – cell constant: $K=0.3131$, precision $\pm 3\%$ per decade) and a platinum resistance probes (Pt 1000 Ω – IEC 751 – Class A).

All sensor electrical signals were conditioned using a data acquisition system (Agilent technologies, Loveland, USA, 34901A) including a multiplexer acquisition module (34901A) and a control command card (34907A) via a RS-232 liaison. Measurements were saved in a text file(".txt") on a PC (PC DELL - ProcessorIntel® Core™2 CPU T5600 @ 1.83GHz - 988MHz of RAM) with a specific software developed on LabView™ 8.6 (National Instrument)

A specific pump command was developed under Labview software to monitor the flow-rate within the derivation loop. Two working modes were used: (i) constant flow-rate and (ii) flow rate sequence. This last one enabled to investigate on-line rheological characterisation during cell cultures. The flow rate sequence is defined by a number of steps, duration and minimal and maximal flow rates.

2.2 Newtonian test fluids

The experimental device was calibrated using three different Newtonian fluids: osmosed water and two glucose solutions. Different temperatures in the range 20 to 40°C were used to ensure a large Reynolds number exploration. The glucose solution was prepared by dissolving hydrated glucose (cerelose, $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$) powder in osmosed water under heating condition. Density and viscosity were controlled for each solution along experiments and in function of temperature.

2.3 Cell culture conditions

Escherichia coli (*E. coli*) is the most widely used microorganism for biological research experiments in microbiology. For this culture, the mutant used was of the strain *E. coli* K12.

Fed-batch cultures were performed in a Chemap AG (20L) bioreactor under highly aerated ($< 3.3\text{VVM}$) and agitated conditions (1500RPM). The temperature was regulated at 37°C and the pH at 6.8 with the addition of 28%v/v ammoniac solution also used as a nitrogen source. Bioreactor contained 2L of initial mineral medium and was inoculated with an important inoculum (around 4L with a biomass concentration close to 60gCDW/L) issued from a previous culture.

During culture, the bioreactor was fed with sterile solutions (glucose solution, mineral medium, pH regulation solutions, anti-foam) using peristaltic pump (Masterflex and Gilson). Carbohydrate feed allowed to control growth rate. Feed flows were calculated in order to control micro-organism activity. Oxygen transfer was performed thanks to air flow and mixing (3 Rushton turbines) as well as a small counter-pressure ($< 100\text{mbar}$).

2.3 Analyses

Broth is sampling along experiment and stored at 4°C in order to analyse system state:

- Cell concentration: spectrophotometric measurement at 600nm (Spectrophotometer Hitachi U-1100, range 0.1-0.6UOD). Cell dry weight, X [gCDW/L] was calculated from an empirical correlation between DO and dry matter.
- Concentration and particle size distribution: laser diffraction analyse (Mastersizer 2000 Malvern Instruments Ltd. range from 0.02 to $2000\mu\text{m}$),
- Cell morphology : optical microscopy (Nikon, x100, in oil immersion, phase contrast mode),
- Rheological behaviour: rheometer (Bohlin C-VOR 200 Malvern Instruments Ltd, geometry: cone-plate $2^\circ/60\text{mm}$, shear rate: 0.1 to 100s^{-1}).

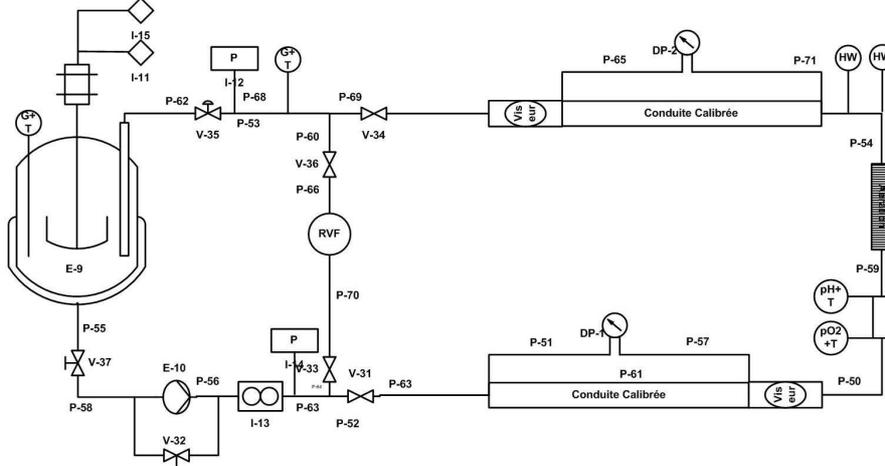


Figure 1: Overview of experimental set-up.

3. IMPLEMENTATION AND RESULTS

3.1 Hydrodynamic identification of the friction curve

Isothermal flow of Newtonian and Non-Newtonian fluids in relatively simple geometries has been studied extensively. **Shah and London, 1978** gave a complete overview of the analytical solutions obtained in laminar flow and semi-empirical correlations for transition and turbulent flow regimes.

$f/2$ is the friction factor and Re is the classical Reynolds numbers defined as follows:

$$\frac{f}{2} = \frac{d_h}{4 \cdot \rho \cdot U^2} \cdot \frac{\Delta P}{L} \quad ; \quad Re = \frac{\rho \cdot U \cdot d_h}{\mu} \quad (1)$$

where ρ and μ are respectively the density and the viscosity, U the mean velocity and d_h the hydraulic diameter of the duct. The friction curve is the representation of $f/2$ against Re . The relationship between the friction factor and the Reynolds numbers for laminar isothermal flow of Newtonian fluids in cylindrical ducts is given by Eq. 2. The parameter, ξ which is the product of the Reynolds number and friction factor stands as the geometrical parameter. It may be theoretical (simple geometries, e.g., circular ducts $\xi=8$, infinite parallel plate $\xi=12$, square duct $\xi=7.113$), semi-theoretical or experimental [**Churchill, 1977**].

$$\frac{f}{2}_{lam} = \frac{\xi}{Re} \quad (2)$$

For transition and turbulent flow regime, numerous semi-empirical correlations can be used (e.g.: Blasius) with the following expression (a, b, c and d: constant coefficients):

$$\frac{f}{2}_{trans} = a \cdot Re^b \quad ; \quad \frac{f}{2}_{turb} = c \cdot Re^d \quad (3)$$

Transition from laminar to transitory regimes and transition from transitory to turbulent regimes occur respectively for critical Reynolds numbers Re_{c1} and Re_{c2} . The friction curve could be described using a unique expression based on Churchill's model (Eq. 4). We can use this expression for laminar, transitory or turbulent flow regimes [**Churchill, 1977**]. This expression is based on the sum of the three regimes contributions as follows:

$$\frac{f}{2} = \left[\left(\left(\left(\frac{f}{2} \right)_{turb}^{n_1} + \left(\frac{f}{2} \right)_{trans}^{n_1} \right)^{n_2/n_1} + \left(\frac{f}{2} \right)_{lam}^{n_2} \right)^{1/n_2} \quad (4)$$

Friction factors and Reynolds numbers issued from experimental measurements with water and glucose solutions are presented on Figure 2. The three flow regimes are easily identified and friction curves exhibit a classical shape for a cylindrical duct. In our conditions, Churchill's equation is modelled using 6 parameters (a, b, c, d, $n_1 = -8$ $n_2 = 12$) which are experimentally identified in laminar, transition and turbulent regimes. The pipe diameter as given by the

constructor is equal to 6mm. Poiseuille law (i.e. the value $\xi = 8$) is used to obtain a better estimation of the hydraulic diameter which is 6.16mm. Values for the parameters a, b, c and d are reported in Table 1, together with values for the critical Reynolds numbers Re_{c1} and Re_{c2} . These ones are determined as the points where differences between experimental data and adjusted law exceed 5%.

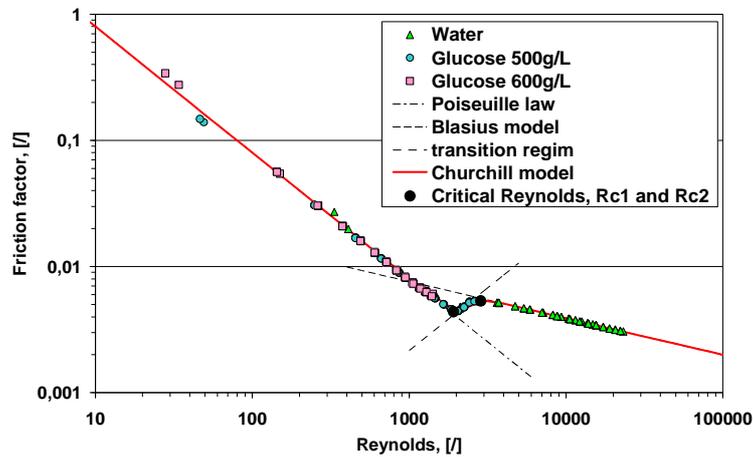


Figure 2 : Friction curves: experimental data and adjusted models (Cylindrical duct, $\varnothing=6/8$ mm, $L=2000$ mm).

Table 1 : Friction curves parameters and critical Reynolds numbers

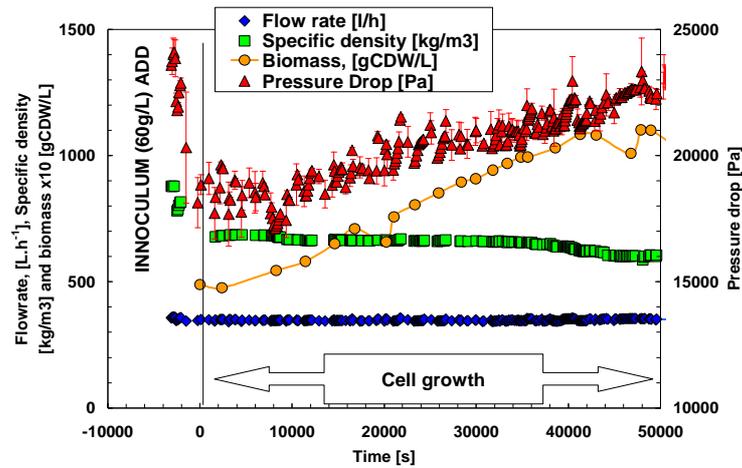
Laminar		Transition		Turbulent		
ξ	Re_{c1}	a	b	c	d	Re_{c2}
8	1920	$2.31 \cdot 10^{-6}$	0.990	0.0566	-0.290	2870

The Churchill model (Eq. 4) using friction factors (Eq. 2 and 3) calculated with the coefficients defined in Table 1 is now our reference curve for on-line rheological measurements.

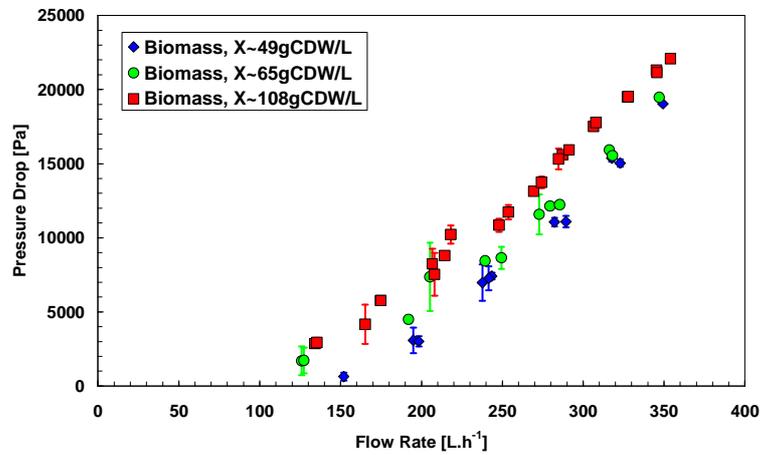
3.2 Cell culture and rheological measurements

E. Coli culture was monitored during 14 hours while biomass concentration increased from 40gCDW/L up to 110 gCDW/L. In figure 3a, the evolution of density and pressure drop were plotted versus time. During the inoculating phase, a sharp decrease of pressure drop and specific density were observed. During culture, pressure drop significantly increased (+ 30%) and density slightly decreased whereas flow-rate was maintained constant (around 350l/h). Meantime, microscopic observations and granulometric analyses revealed that the mean diameter was constant, close to 1.05 μ m, with a spherical shape.

Biomass concentration and biological activity of microorganisms were supposed to interact with physical properties such as density and viscosity. To get further information, flow-rate sequences as previously defined, enabled to investigate the rheological behaviour of the fermentation broth at defined time. In figure 3b, pressure drop versus flow-rate curves were plotted at selected time corresponding to different biomass concentrations. Separate curves demonstrated that the apparent viscosity evolved along cell culture. The increasing pressure drop observed indicated an increase of apparent viscosity in agreement with the evolution of biomass concentration.



3a



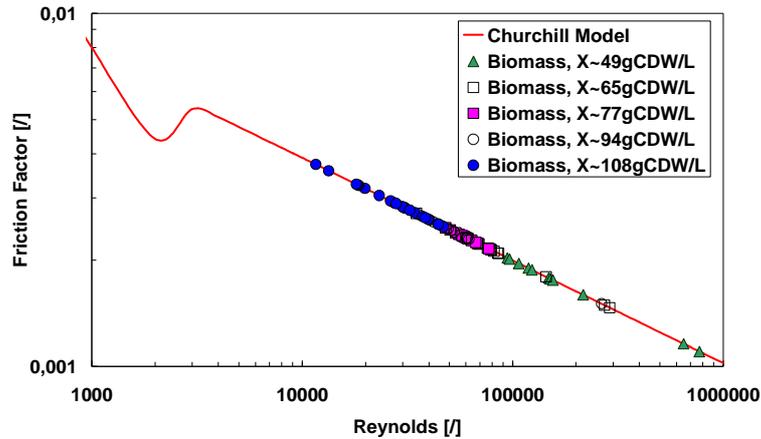
3b

Figure 3: Evolution of flow-rate, density and pressure drop versus time (Fig. 3a) and DP-Q curves for selected biomass concentrations (Fig. 3b).

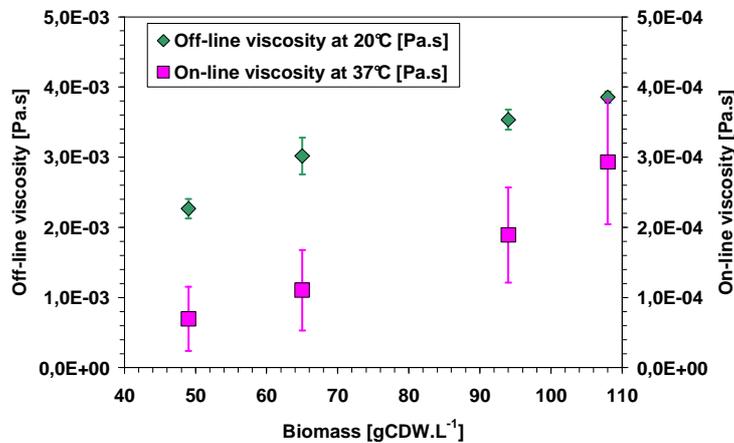
Pressure drop measurements lead to determine the friction factor in agreement with Eq.1. Using established friction curve, a Reynolds number was calculated for each experimental point (Figure 4a) and the apparent viscosity at 37°C was deduced. It highlighted that the on-line rheological behaviour of cell broth could be determined and remained Newtonian.

Laboratory measurements (off-line) of rheological behaviour (at 20°C) confirmed that cell broths exhibited a Newtonian behaviour which was dependant on biomass concentration. In figure 4b, on-line and off-line apparent viscosities were compared. Both curves exhibited the same tendency but strongly differed in term of magnitude. Several assumptions could be formulated to explain this difference: (i) thermal dependency of apparent viscosity, (ii) biological activity and (iii) volume gas fraction (aeration).

Thermal dependency of apparent viscosity could not explain such differences between on-line and off-line apparent viscosities. Density measurements gave insight on the importance of gas fraction (gas retention) within broth. Overall these results demonstrated the need of on-line rheology in order to achieve and quantify reliable information about the rheological behaviour of cell broths inside the bioprocess.



4a



4b

Figure 4: Evolution of friction factor at different flow-rate for selected biomass concentration (Fig 4a) and comparison on-line and off-line apparent viscosity versus biomass concentration (Fig 4b).

4. CONCLUSION

Rheological behaviour of culture broth stands as a fundamental parameter in bioprocess performances. In this study, our objectives were to develop and identify an experimental tool enabling on-line rheology and to validate measurements with a cell culture. This work describes implementation and results obtained with an experimental on-line rheological device mounted on a bioreactor. Description of the set-up and experimental calibration with well-defined Newtonian fluids are presented. An example of a cellular culture (*E. coli*) is then proposed enlightening the influence of biological activity on rheological behaviour and the need for on-line measurement.

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