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Abstract:
Rheological behaviour of culture broth stands as a fundamental parameter in bioprocess performances because it affects simultaneously the heat and mass transfer as well as the flow pattern. On-line measurements of rheological behaviour are hardly compatible with the operating condition with respect to accurate and stringent conditions imposed by cell culture strategy. Our scientific and technical objectives are (i) to develop and identify an experimental device enabling on-line rheometry and (ii) to discuss and compare on-line and off-line measurements. In this aim, a bioreactor was equipped with a derivation loop including a specific on-line rheometric device as well as additional physical and biological measurements (specific density, mass flow rate, electrical conductivity, pH, pO_2 and temperature) during microbial cell cultures. In a first time, friction curves of calibrated ducts were established with Newtonian and non-Newtonian shear-thinning fluids. In a second time, axenic cultures with two microorganisms (bacteria and yeast exhibiting different sizes) were investigated in pure oxidative culture in order to produce biomass under high cell concentrations: ~ 40 to 110 g_{CDW}/l for *E. coli* (bacteria) and ~ 75 to 105 g_{CDW}/l for *Y. lipolytica* (yeast). Cell broths exhibited Newtonian behaviour for *E. coli* and shear-thinning behaviour for *Y. lipolytica*, which were both dependant on biomass concentration. On-line and off-line rheological measurements are consistent for *E. coli* and *Y. lipolytica*, but significantly differed. On-line estimated viscosity appears higher than off-line apparent viscosity. Several assumptions in relation with microorganism physiology and metabolism (size, morphology, surface properties, concentration, biological activity) could be formulated in agreement with scientific literature. On-line rheology brings new insight to investigate complex interaction between physical and biological phenomena.

Zusammenfassung:
Das rheologische Verhalten von Kulturen stellt einen entscheidenden Paramter in der Leistungsfähigkeit von biologischen Prozessen dar, da es sowohl die Wärme und Massenübertragung, als auch das Flussverhalten beeinflusst. On-line Messungen des rheologischen Verhaltens sind nicht mit den exakten Arbeitsbedingungen die für die Zellkulturenstrategie nötig sind, vereinbar. Unsere wissenschaftlichen und technischen Ziele sind (i) die Entwicklung eines experimentellen Instruments zur on-line Rheometrie und (ii) on-line und off-line Messungen miteinander zu vergleichen. Dafür wurde ein Bioreaktor mit einem Rohr in Derivation mit einem spezifischen on-line Rheometrie Instrument und zusätzlichen physikalischen und biologischen Meßgeräten (spezifische Dichte, Durchflussrate, elektrische Leitfähigkeit, pH, pO_2 und Temperatur) ausgestattet. Zunächst wurden Reibwertkurven erstellt mit kalibrierten Mitteln mit Newtonsches und allgemeinen Nicht-Newtonsches strukturviskosen Flüssigkeiten. Im zweiten Schritt wurden axenische Kulturen von zwei Mikroorganismen (Bakterien und Hefern verschiedener Größen) in reiner oxidativen Kultur untersucht um Biomasse mit hoher Zellkonzentration zu erzeugen: ~ 40–110 g_{CDW}/l für *E. coli* (Bakterien) und ~ 75–105 g_{CDW}/l für *Y. lipolytica* (Hefe). Das Kulturmedium zeigte Newtonsches Verhalten mit *E. coli* und strukturviskoses Verhalten bei *Y. lipolytica*, wobei beides von der Biomassekonzentration anhing. On-line und off-line rheologische Messungen sind consistent für *E. coli* und *Y. lipolytica*, aber unter scheiden sich deutlich. Die geschätzen Werte für Viskosität sind höher wenn on-line gemessen. Verschiedene Annahmen über die Physiology des Mikroorganismus und den Metabolismus (Größe, Morphologie, Oberflächeneigenschaften, Konzentration, biologische Aktivität) konnten im Einklang mit der Literatur formuliert werden. On-liner Rheologie ermöglicht neue Einblicke in die Untersuchung der komplexem Interaktion zwischen physikalischen und bilogischen Phänomenen.

Résumé:
Le comportement rhéologique des moûts de fermentation représente un paramètre fondamental pour le contrôle et l'intensification des bioprocédés car il affecte simultanément les transferts de chaleur, de matière et de

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behaviour [8] and it may not be excluded that these wall properties evolved when the cell culture conditions are not respected, as occurs when a sample is taken. Supernatant properties can also affect the broth behaviour when cells produce metabolites or extracellular polymeric substances [17]. But this third assumption is not true here as the supernatant of broth cultures is Newtonian with very low viscosity. However, all these works are usually realised off-line and do not take into account the impact of biological activity and the presence of gas volume fraction (aeration/respiration) in broth. This point needs to be taken into account as shown by Barbot et al. [28] who explored the influence of respiration in the case of activated sludge under aerated condition within a bioreactor. In their experiment, rheological behaviour was evaluated using on-line torque measurements. They observed that inhibition of respiration by sodium azide addition leads to an evolution of the rheological behaviour. However, the comparison between activated sludge and axenic cell cultures is limited considering differences between experimental conditions and broth composition. But this demonstrates that biological activity is an additional difficulty for broth characterization and has to be considered during the measurements.

4 CONCLUSION

Rheological behaviour of broth stands as a fundamental parameter that could limit bioprocess performances. Therefore axenic culture of microorganism in bioreactor involves culture strategy with aeration as a necessary and obligatory parameter. All the complexity related to on-line monitoring of rheological behaviour results in the setting up of an adapted tool with correct measurement in respect of cell culture conditions. In the case of torque measurement, the variation of agitation speed and aeration flow rate to allow correct flow regime can hardly be achieved (risk of disrupt the system) except in robust biopro - cess (wastewater treatment).

In this study, our objectives were to investigate on-line and off-line physical measurement with a new device during axenic cell cultures under high biomass concentration. This dedicated and innovative experimental set-up was developed and was calibrated with well-defined Newtonian and non-Newtonian fluids. Two microorganisms (*E. coli* and *Y. lipolytica*) with different cell size were cultivated. The physical measurements showed different results: (i) the impact of cell concentration on pressure drop, (ii) the contribution of the gas phase in the broth, (iii) the possible impact of difference between off-line and on-line shear rate ranges that can be explored and (iv) a setting up of caution to use properly our experimental set-up and to estimate the on-line viscosity equivalent to a homogenous liquid medium. Our works demonstrated the need and usefulness of on-line rheology measurements to achieve and quantify reliable information about the dynamic rheological behaviour of cell broths during cultures. Our researches bring new insights on the dynamical interactions between physical and biological phenomena in bioprocess which will be useful to progress in the evaluation and the control of their performances.

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