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Use of on-line flow cytometry for the characterization of physiological behavior in stress conditions during the bioprocess

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Backgroung

Microbial cell population heterogeneity is now recognized as a major source of issues for the development and optimization of bioprocesses. Even if single cell technologies are available for the study of microbial population heterogeneity, only a few of these methods are available in order to study the dynamics of segregation directly in bioreactors. Flow cytometry is a very powerful tool for the follow up of physiological properties of microbial cells in process-related conditions. In this context, specific interfaces have been developed in order to connect flow cytometer (FC) directly on bioreactor for automated analyses. In this work, we propose a simplified version of such interface and demonstrated its usefulness for multiplexed experiments.

Results

We propose to use a benchtop Accuri flow cytometer as a basis for the design of an automated FC (figure 1). Fluid displacement is ensured by peristaltic pumps, facilitating the set-up of an interface with a bioreactor since no pressurization of the sample is needed. The development of previous systems was indeed impaired by the need to maintain pressure at the level of the sample unit. Sample dilution and staining with propidium iodide (PI) is carried out in line in the tubing between the FC and the bioreactor.

This automated FC system has been tested for the follow up of the dynamics of an *E.coli pfis::gfpAAV* fluorescent bio-reporter and its PI uptake, correlated with membrane permeability. This bioreporter is composed of a *fis* promoter, a growth dependent promoter-indicator of the nutrient status of cells, fused to a gene expressing an unstable variant of GFP. The results obtained showed that the dynamics of the GFP synthesis is complex and can be attributed to a complex set of biological parameters, i.e. on one hand the release of protein to the extracellular medium and its uptake modifying the activity of the *fis* promoter, and on the other hand the stability of the GFP molecule itself that can be attributed to the protease content and the energy status of the cells. Segregation in the membrane permeabilitity has been noticed.

Conclusion

This work demonstrates that a simplified version of on-line FC can be used at the process level for the investigation of the dynamics of complex physiological mechanisms.

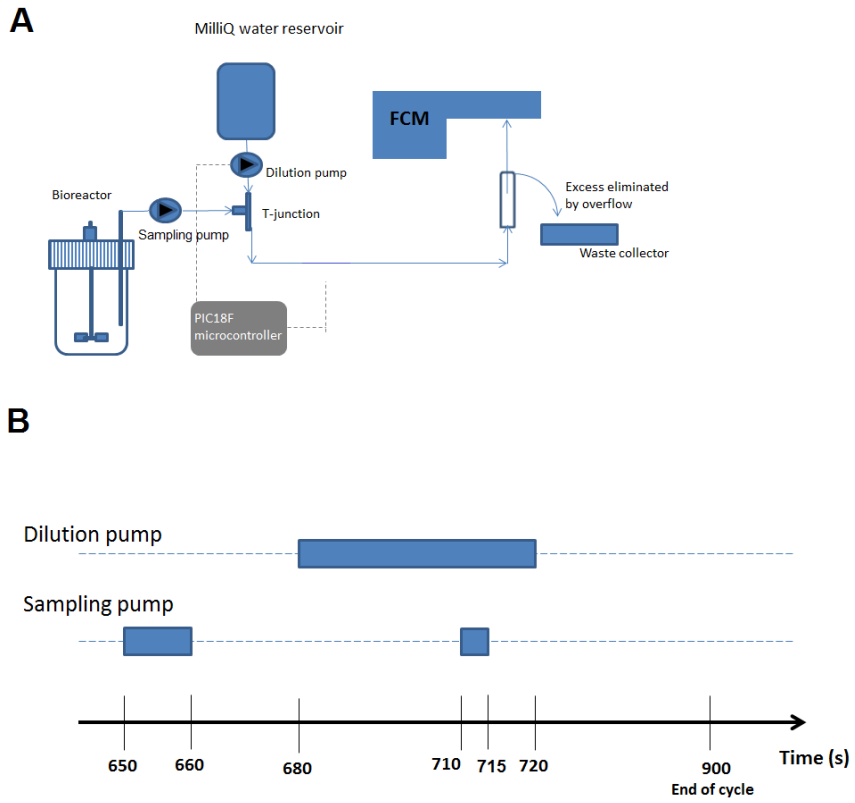

Figure 1. A : Scheme of the interfacing system between the bioreactor and the FC B : Sequence of activation of the dilution and sampling pump. First activation of the sampling pump to fill the line and second activation simultaneously with the dilution pump to reach an appropriate dilution of the sample before FC analysis