E.coli prpoS::gfp strain as biosensor of glucose heterogeneity inside industrial bioreactors

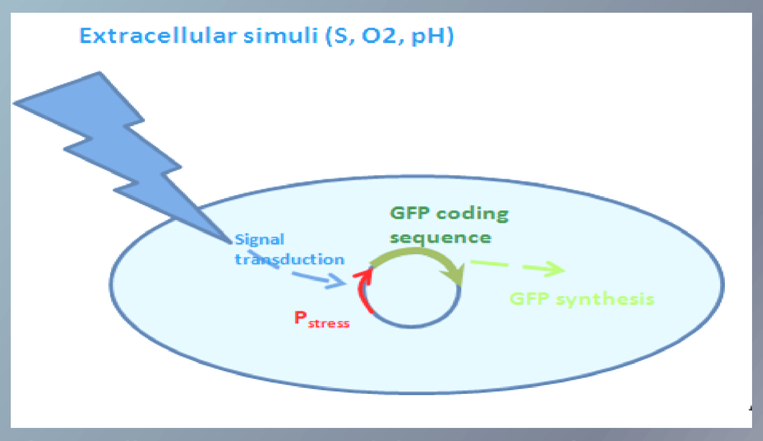
Brognaux A., Delvigne F., Thonart P.

Unité de Bio-industries- Gembloux Agro Bio-Tech - Université de Liège

Objectives

Escherichia coli is a microorganism widely used in the industry for the production of recombinant proteins. The performances obtained at the laboratory level are not reproducible at a large scale. Actually, the mixing operation is not efficient enough: gradients of glucose and oxygen appear when operating in fed-batch mode (addition of glucose during the culture). These gradients cause adverse impacts on the production of biomass and recombinant protein. The aim of this work is to use the microbial population as biocaptor of the encoutered stress inside heterogeneous industrial bioreactors to better scale-up and regulate these reactors

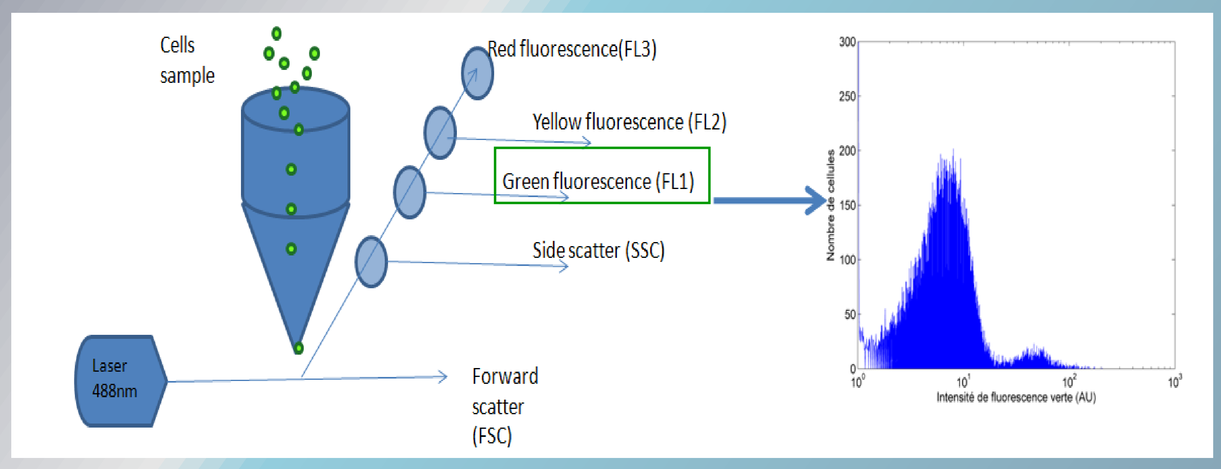
Material and methods



The coding sequence of the Green Fluorescent Protein (GFP) is introduced after a specific stress promoter in a plasmid. This one is inserted in *Escherichia coli*. When these cells are submitted to a given stress condition (depending on the function of the promoter), GFP synthesis is induced and accumulated into the cytoplasm

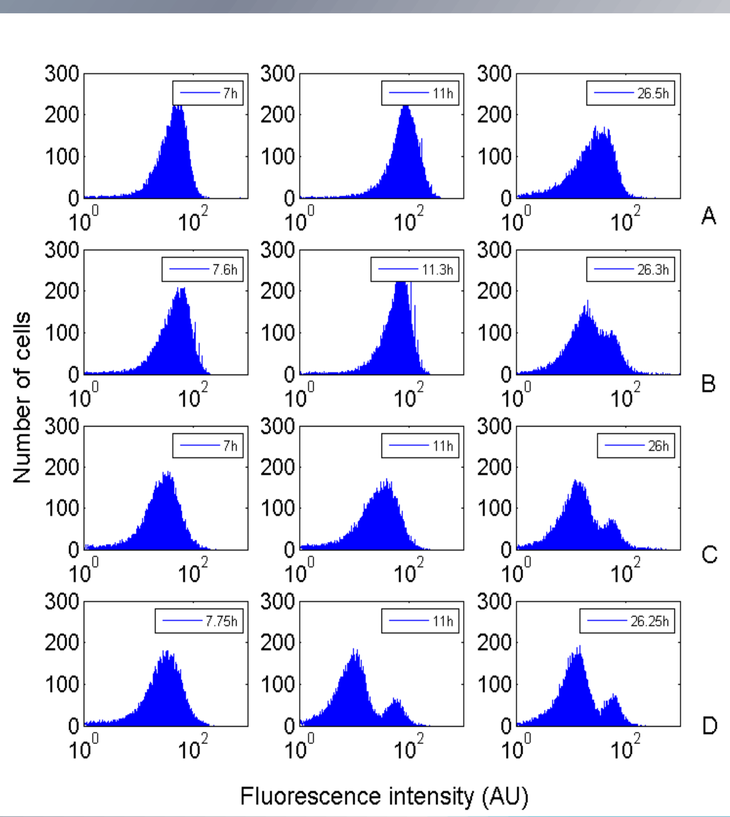
The corresponding stressed cells becomes fluorescent!

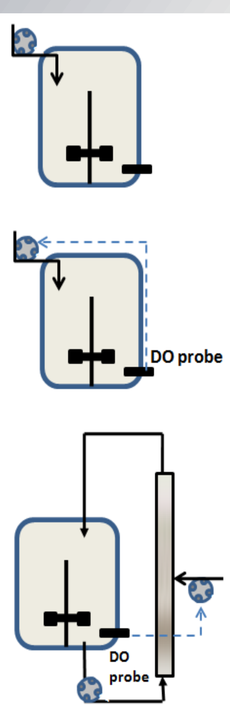
Fluorescence analysis by flow cytometry



Flow cytometry allows the green fluorescence analysis at the single cell level:obtained results are frequency histograms of fluorescence-intensity in the microbial population . So, it is possible to link the GFP content among the microbial population with the bioreactor heterogeneity (stress condition)

Results





A — Exponential feeding in a well-mixed bioreactor

B — Glucose feeding accordind to dissolved oxygen in a well-mixed bioreactor

C — Glucose feeding accordind to dissolved oxygen in an heterogeneous bioreactor called scale-down bioreactor

D

Lesser mixing efficiency: more heteroheneous extracellular environement

Duration of the culture

Cultures with *Escherichia coli* prpoS::gfp strains have been carried out in several types of bioreactors that have different mixing efficiency.

The rpoS gene is a gene of the general stress response, mainly induced at the entrance to stationary phase (during a lack of glucose).

The GFP gene has been introduced after the rpoS promoter. The fluorescence has been monitored at the single cell level by flow cytometry.

During variations in extracellular glucose, there is a decrease of the total measured fluorescence caused by a segregation of the population in two subpopulations.The intensity of the segregation, as well as its time of appearance during the culture can be related to the bioreactor mixing efficiency.

Conclusion

prpoS::gfp strains can be used as biosensors of the heterogeneity of glucose encountered inside industrial reactors.

Potential application and key benefits

These strains could be used to validate a fed-batch regulation (addition of glucose) at the industrial level.